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THE SECRETION OF GASTRIC JUICE IN HEALTH AND DISEASE

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I. METHODS: *1. Methods of securing the gastric secretion.* The advances in our knowledge of gastric secretion run parallel with the development of new experimental methods and the degree of intelligence with which these methods are applied, experimentally and clinically. Reaumur and Spallanzani introduced food and sponges in perforated metal or wooden capsules into the stomach of man, birds and fishes. These capsules were recovered by means of attached strings, by vomiting, or by passage per rectum. By diligent application of these simple methods Reaumur and Spallanzani demonstrated *a*, that gastric digestion is a process of chemical solution rather than mechanical trituration; *b*, that gastric juice is acid; *c*, that it prevents putrefaction; and *d*, that it digests food in vitro.

The next step in advance was a chemical one, taken by Prout and Tiedemann and Gmelin. They secured gastric juice or gastric content by killing their animals after introducing pebbles and other indigestible material into the stomach, or at the height of digestion of a meal, and proved by adequate methods that the acidity of the gastric juice is due to free hydrochloric acid.

Next came data from clinical and experimental gastrostomy. While Beaumont was not the first to note gastric digestion and secretion in human gastric fistula cases, he was the first to make serious use of such a case to advance our knowledge of gastric physiology and pathology. Moreover, his classical research on Alexis St. Martin became the most important impetus to the work on gastric physiology in the 19th century. Beaumont secured pure gastric juice, but the limited chemical examination of this juice was made by others. Beaumont concluded, however, that gastric juice contains other food solvents besides the HCl. The substance hinted at was pepsin, discovered by Wassman six years

later (1839). Beaumont recorded that irritating condiments, excess of alcohol, febrile states, anger and fear, etc., decrease gastric secretion, facts that have been confirmed and extended by later workers. But Beaumont is also partly responsible for promulgating and perpetuating two erroneous views concerning gastric secretion, viz., that there is no secretion of gastric juice in the absence of food in the stomach, and that foods or indigestible solids induce secretion by their mechanical action on the gastric mucosa. But the blame for the perpetuation of these errors rests heavier on the followers than on the master, Beaumont. Since the publication of Beaumont's great work nearly ninety years ago important contributions have been made to gastric secretion on many other human gastrostomy cases, both in health and disease.

Simple gastrostomies on experimental animals quickly followed the publication of Beaumont's observations in 1833 (Bassow, Blondlet, 1842), but these led to no discovery of fundamental importance except the demonstration of Bidder and Schmidt that the HCl is actually produced by the gastric glands. The next important step, again a new method, was taken by Heidenhain in making the isolated pouch, or little stomach separated from the main stomach, so that pure gastric juice was for the first time obtained in the experimental animal parallel with normal digestion. Heidenhain's pupil, Pavlov, in the early nineties, made the important addition to the Heidenhain operation of so cutting the gastric walls that most of the extrinsic and intrinsic nerve connections were retained in the isolated gastric pouch. In this way Pavlov and his pupils were able to begin the analysis of the chemical and the nervous factors of the gastric secretion. The later development of Pavlov's research in the direction of conditioned reflexes deal with the central nervous system rather than with gastric functions.

The stomach tube,—Fractional analysis. The discovery and application of the principle of the stomach tube or stomach pump appears to go back into antiquity (Garrison). In modern medicine the stomach tube was first used for tube feeding and for gastric lavage (Monroe, Physick, Hunter, Kussmaul). But shortly after Kussmaul's first publication on the subject in 1867, Leube and Külz made use of the stomach tube for the purpose of securing gastric content and analysis of gastric secretion. As quoted by Maly, these investigators introduced the principle of fractional analysis, that is, removal of small portions of the test meal at fixed intervals, in order to follow the secretion process by the variations in acidity and pepsin content. This method was also followed by Schüle, who in addition showed that the appetite secretion of gastric

juice may be studied in intact persons by emptying the stomach, chewing palatable food without swallowing for fixed periods, and withdrawing the juice secreted. Following the lead of the great German gastroenterologists, most of the clinical work on gastric secretion during the last fifty years has been done by the less satisfactory method of withdrawing the entire gastric content at a fixed interval (usually one hour) after ingestion of the test meal. This procedure affords no control on the continuous secretion before ingestion of the food, and no adequate information on the secretion rate following the evacuation of the meal. The great body of data in the clinical literature on gastric juice secured by this method is therefore of doubtful value, even for diagnostic purposes. To be sure, Ewald, Boas, Reichmann and others attempted fractional studies by giving the same test meal on succeeding days or by removing the entire gastric content at different intervals after ingestion.

The large caliber and relatively inflexible catheter usually employed as stomach tube is too uncomfortable to retain in place for any considerable period, and too difficult to swallow so that passing this tube into the stomach at 15- or 20-minute intervals is out of the question. The motor and secretory disturbances induced reflexly by this procedure would vitiate the results. Delicate flexible catheters that could be swallowed with ease and retained in place with comfort over long periods have been used from time to time and were recommended for the study of gastric content by clinicians both in this country and in Europe. Thus in 1893 Gross reported the use of a soft nelaton catheter for gastric analysis. But these methods received little attention until about a decade ago when the modern method of fractional gastric analysis was introduced by Ehrmann, Ehrenrich and Ettinger, in Germany, and a little later by Rehfuss and his co-workers in America.

The adaptation of the duodenal catheter of Einhorn for the purposes of recovery of the gastric content, continuously or at short intervals, is a noted advance as regards the study of gastric secretion in man both in health and disease. The main shortcoming of the fractional method in connection with the test meal is due to the variation in degree of acidity of the gastric content in different regions of the stomach (Gorham, Kopeloff, White, Fitz).

Taylor has described an ingenious device for securing pure gastric juice on "sham" feeding in normal infants. The stomach tube is passed through a rubber tube, the latter ending in the esophagus. This outer tube is connected with a suction apparatus so that the milk or other liquids swallowed are sucked back through this outer tube before reaching the stomach.

The tube (nelaton catheter) can be swallowed by most people without difficulty and retained indefinitely with no retching and little or no salivation. Since this stomach tube cannot be pushed into the stomach it cannot be used in refractory individuals (insane, hysterical subjects), and because of its diameter, the tube cannot be used to recover gastric contents containing foods not thoroughly masticated.

The scientific value of much of the experimental and clinical data on gastric secretion is greatly decreased by the failure to secure controls on the continuous secretion.

Extracts of the gastric gland. The study of gastric secretion by extracting the mucosa is of very limited value, except as an aid in localization of the pesin-producing glands. So far, this method has yielded nothing conclusive on the question of place and mode of origin of the acid and ferments of the gastric secretion.

2. Method of determination of gastric acidity. The literature on methods of measuring the acidity of gastric juice is considerable.

The direct titration with NaOH was started by Sazabo, and developed into the present form (using dimethyl-amin-aso-benzol) by Köster, Mintz, Mörner and Tröpfer. The controversy concerning the method has been as to which of the numerous indicators used yields the most accurate result, and whether the figures for free HCl are not too high, the addition of the alkalis liberating some of the combined acid. To overcome this last objection Sörenson introduced the colorimetric method. The principle of this method is the selection of a series of indicators that change in color at definite degrees of acidity. Sholl has recently worked out a series of indicators and stable color standards permitting direct reading of the approximate pH of the gastric juice.

Theoretically, the gas chain method yields the most accurate information of the true acidity of a solution, and this method has been used by a number of investigators on the gastric juice (Michaelis and Davidson, Christiansen, Menten, McClendon). McClendon has devised a hydrogen electrode which can be introduced into the stomach or duodenum by means of a catheter. This permits the determination of gastric and duodenal acidity during digestion without removing any of the gastric content, Christiansen has reported a thorough-going comparative study and critique of these several methods of determining gastric juice acidity. On normal human gastric juice the standard titration and the gas chain method yield figures so nearly identical that the titration method is sufficiently accurate for all clinical and for most research purposes.

3. Methods of measuring the pepsin concentration. Since the chemical structure of pepsin is not yet known all methods of determining the pepsin concentration in gastric juice are only relative. The methods are all based on the rate of solution of some protein. Grützner modified the older method of Brücke by using carmine-stained fibrin, the rate of digestion being measured by the rapidity of liberation or solution of the carmine. Jacoby used the rate of clearing of a solution of ricin as a measure of pepsin concentration. Fuld and Levinson used edestin. Gross used casein, Hammarschlag egg albumen, the rate of digestion being determined in each case by the amount of protein remaining to be precipitated by sodium chloride, sodium acetate and Esbach's reagent, respectively. Huppert and Schütz used the rate of production of secondary albumoses as a measure of pepsin, and on the basis of this method they formulated the law that the rate of pepsin digestion is proportional to the square root of the pepsin concentration, a generalization that has, at the most, only a limited application (Oppenheimer). Glässner uses the rate of hydrolysis of globin.

The simplest, and for clinical purposes at least, quite the most satisfactory method is that of Mett, the pepsin concentration being calculated from the rate of digestion of egg white coagulated in glass tubes. Pepsin concentration has also been measured by the changes in the viscosity (Spriggs), and the electrical conductivity (Sjöquist, Oker-Blom) of protein solutions.

II. Secretion of gastric juice in the lower animals: 1. Gastric secretion in the invertebrates. Gastric juice, with its free hydrochloric acid and specific ferments (pepsins) appears to be confined to the vertebrate animals. No free mineral acids or proteolytic ferments requiring free acids for their digestive action have so far been conclusively demonstrated in the stomachs of any invertebrate group. Acid reaction of gastric contents or secretion have been noted by many observers, and some have assumed this to be due to hydrochloric acid. Later and more careful work seems to show that this acidity is due either to *acid salts* or to acid saliva, and not to acid gastric juice, although Griffith quotes Fredericq as having reported that the gastric juice of Mya contains hydrochloric acid. Bodansky and Rose studied the digestive action of gastric mucosa extracts of certain coelenterata (Stomolophus, Physalia) on gelatin. They conclude that pepsin is present, since the extract liquefied gelatin in an acid medium, the optimum acidity being pH 3.0, or the same as for mammalian pepsins.

The free mineral acid (H_2SO_4) in the so-called salivary glands of some molluscs is not accompanied by pepsins or pepsin-like ferments. The digestive significance of this salivary mineral acid is therefore not analogous to the hydrochloric acid of the vertebrate gastric juice. There appears to be, at times, free mineral acids in the digestion vacuoles of some of the unicellular animals, but in these animals no proteolytic action goes on until the free mineral acid is fixed or neutralized. The rôle of this acid vacuole secretion is therefore supposed to be the *killing* rather than the *digestion* of the ingested bacteria and spores. The literature is adequately reviewed by von Fürth, Griffith, and Jordan. Invertebrate digestion is essentially pancreatic and intestinal.

The biological significance of this apparently uniform absence of gastric secretion (HCl, pepsins) in the invertebrates, and its normal presence in the entire vertebrate phylum is by no means clear. But it would seem that gastric juice is a comparatively late addition to the animal digestive process. It might be worth the while to reinvestigate the gastric secretion in the groups supposed to be the nearest relatives of the primitive vertebrates.

The late evolution of gastric juice may have some significance in the frequency of chronic achylia in normal persons and the apparent absence of digestive disorders in chronic or temporary achylias not complicated by motor disturbances of the gut.

There appears to be a continuous secretion of the digestive juices in most of the invertebrate groups. The question of nervous and chemical control of these secretory processes does not appear to have been investigated.

2. Gastric secretion in the fishes. There is a considerable literature on gastric secretion and gastric digestion in fishes, beginning with Spallanzani (1783). This is adequately reviewed by Sullivan and by Beidermann. The problems attacked are the nature of the acid or acids, and the proteolytic ferments, and the place of formation of the acids and the ferments. The question of the mechanisms of secretion of the gastric juice has scarcely been touched in the fishes, although some species of elasmobranchs would be suited for gastrostomy and Pavlov pouch operations. Sullivan concludes that in the elasmobranchs both the pepsin and the hydrochloric acid is secreted by the cells in the gastric sac, that is, the region corresponding to the fundus of the mammalian stomach. In the elasmobranchs the fundus glands are not differentiated into the chief and the parietal cells. According to Sullivan the pyloric mucosa secretes neither pepsin nor HCl.

It seems established that the gastric glands in all groups of fishes secrete HCl and pepsins. Some observers have also reported sulphuric and organic acids. In fact, Weinland and Van Herwerden report that the gastric juice of the shark contains only organic acids. But the gastric secretion in fishes seem to differ from that of the mammals in three particulars: *a*, the high concentration of HCl; and *b*, the rapid action of the fish pepsin at low temperatures; *c*, the high optimum acidity for the action of the fish pepsins. Richet reported a gastric acidity in selachians of from 0.6 per cent to 1.4 per cent. Young reported an acidity of 0.84 per cent. Sullivan reports that in the fasting shark the stomach reaction is practically neutral. His observations were therefore made on the gastric content at the height of digestion; under those conditions he found the total acidity as high as 1 per cent HCl, and the free or physiologically active acidity as high as 0.6 per cent HCl. A Russian observer (Svolima) has recently reported an acidity of 1.6 per cent HCl in the shark's stomach. This observer appears to have secured pure gastric juice by means of a gastric fistula. Wieland reports the secretion of an alkaline gastric juice in the skate (Raja).

The high acid optimum (0.5 per cent free HCl) and the rapid proteolytic activity of fish pepsins at low temperatures are interesting differences from mammalian pepsins that deserve more detailed investigation. Using artificial gastric juice (extract of mucosa) of fishes and ordinary commercial pepsin, Bodansky and Rose found the optimum for fish pepsins to be the same as for mammalian pepsins or pH 3.0. The reports are conflicting as to the presence of rennin and lipase in the fish gastric juice. Bodansky and Rose found rennin present in the gastric mucosa extract of some species of fish, absent in others. Some observers report a high acidity in the fasting fish stomach. This may be either appetite secretion or continuous secretion.

Riddle introduced Mett's tubes directly into the stomach of fishes and determined the variations in the rate of the gastric digestion with the season and the temperature. He reports that temperature and season being the same, the gastric digestion in fishes is much more rapid than in frogs and turtles.

3. Gastric secretion in the amphibians, reptiles and birds. Our knowledge of the gastric secretion in these animal groups is very fragmentary, despite the fact that the earliest experimental work on gastric secretion and digestion appears to have been done on birds (Reaumur, 1752).

Spallanzani found an abundance of clear acid (to taste) fluid in the stomach of chickens, geese and falcons.

Klug reported that the gastric mucosa of geese contained pepsin, and later Paria-Mall and Braimaier found that the pepsin content of the gastric glands is greatly reduced during digestion and rapidly restored in the first hours of fasting, despite the evidence of continuous secretion in the fasting stomach (Teichmann). The difference in the pepsin content of the fasting and the digesting gastric mucosa in birds appears much greater than in the mammal.

Pepsin appears to be absent from the mucosa of the crop, but proteolytic digestion may go on in the crop as a result of gastric juice being forced backward into this organ. The frequent presence of bile in the gastric juice of the empty stomach suggests duodenal antiperistalsis induced as an acid reflex similar to that in mammals.

Karpov made a gastric fistula in the goose and secured gastric juice by sham feeding. This gastric juice showed an acidity of 0.30 per cent HCl, but was very poor in pepsin. Collip reports that extracts of the chicken proventriculus and duodenal mucosa as well as extracts of the thyroid cause secretion of gastric juice in the chicken. In chickens with esophageal fistula, forced swallowing of water also induces gastric juice secretion. Kaskowski, working on pigeons provided with gastrostomy, states that histamine given by mouth or intravenously does not induce gastric secretion, while hypodermic or intramuscular injections of the drug are effective. Very large doses of histamine introduced directly into the intestine also induce gastric secretion.

Langley described the usual histological changes (decrease in granules) in the gastric cells of the snake during digestion. Langley obtained little or no pepsin from the mucosa of the pyloric region.

Swiecicki reported an abundance of pepsin in the esophageal mucosa of the frog, and this appears to be substantiated by Langley and others both by histological and biochemical methods. The granules of the gastric and esophageal cells are diminished during digestion, but the restoration of the granules begins before the gastric digestion is complete. The HCl appears to be secreted by the gastric glands only.

Riddle put Mett's tubes directly into the stomach of frogs, salamanders and turtles and found that the digestion rate was at the minimum in January-March (hibernating season), and at its maximum in midsummer. Riddle's results indicate a continuous secretion of gastric juice in the stomach during the hibernating season, as the method of investigation probably eliminated the appetite secretion factor, if this mechanism is present below the mammals.

The problems of nervous and hormone control of gastric secretion have not been adequately studied in these groups. Boenheim appears to have demonstrated secretion of HCl by the excised gastric mucosa of the frog under the influence of pilocarpin.

III. SECRETION OF GASTRIC JUICE IN NORMAL MAMMALS. *A. Contents of the "empty" stomach.* The normal stomach, empty of food, usually contains some fluid and mucus. This fluid of the empty stomach is made up of *1*, gastric juice; *2*, saliva; and *3*, duodenal contents (bile, pancreatic juice and succus entericus). The chemistry of the contents of the empty stomach depends on the relative preponderance of these three factors. The duodenal juices are frequently absent from the empty stomach. If the continuous gastric secretion is unusually rapid, the empty stomach content may approach the composition of pure gastric juice.

The quantity of empty stomach content varies greatly even in apparently healthy persons. Verhagen reported as high as 50 cc. with an average of 10 to 25 cc.; Moritz found 24 to 64 cc.; Rehfuss, Bergheim and Hawk find 30 to 180 cc. to be within the normal range. Fowler and Zentmire found in ninety healthy women an average gastric content of 50 cc. The average of several hundred observations on the author's three gastric fistula cases (two adult men and a girl of twelve all having cicatricial stenosis of the esophagus) is 30 cc., with variations of 5 cc. to 120 cc. We have occasionally found normal subjects with the stomach literally empty in the morning before breakfast. We found on Mr. V. on the whole the content of the stomach greater in the morning before breakfast than at noon before lunch. It was also greater in the summer than during the winter months. These variations are probably related to gastric tonus and motility rather than to the rate of continuous gastric secretion.

The acidity of empty stomach contents may vary from almost zero up to full gastric juice acidity. In all three gastric fistula cases the figures ran as follows:

	FREE ACIDITY PER CENT			TOTAL ACIDITY PER CENT		
	Low	High	Average	Low	High	Average
Mr. V.....	0.10	0.35	0.18	0.15	0.40	0.23
Mr. E.....	0.09	0.36	0.20	0.20	0.42	0.25
Miss C.....	0.08	0.40	0.22	0.13	0.45	0.26

The pepsin concentration of the empty stomach content may be higher but is usually a little lower than that of pure gastric juice.

B. The continuous secretion of gastric juice by the fasting stomach in normal men and animals. Beaumont and Pavlov appear to be mainly responsible for the view that in the absence of food or so-called psychic stimuli, the gastric glands are quiescent. The corollary to this, namely, that secretion of gastric juice in the absence of food or "psychic factors" is a pathological manifestation has had wide acceptance among clinicians. Both of these views are erroneous. It has been shown that a more or less continuous secretion of gastric juice in the absence of food and evident psychic factors is a normal phenomenon both in man and experimental animals. The complete rest of the gastric glands is an exceptional state in the healthy individual and does not occur for long periods, even in prolonged fasting.

We have made observations on three gastric fistula cases (normal persons except for cicatricial stenosis of the esophagus). On one of these subjects, Mr. V., the observations extended over six years. In this individual the continuous secretion varied from 10 cc. to 60 cc. per hour. A continuous secretion was practically always present also in the other two cases, but the observations on them were not so extensive. If the gastric juice is removed from the stomach every 10 or 15 minutes the total secretion for the hour is greater than if it is removed at longer intervals. This may be due to passage of some of the secretion through the pylorus.

The continuous secretion is true gastric juice, containing HCl and pepsins. The percentage of acidity varies directly with the secretion rate, but is usually less than that of appetite gastric juice.

It may be objected that persons with gastrostomy and cicatricial esophageal stenosis are not normal, since they have a rubber tube in the stomach cavity. But Pavlov has shown that mechanical stimulation of the gastric mucosa is probably not a stimulus to gastric secretion, and it is difficult to imagine how a cicatricial stenosis of the esophagus not involving the vagi might induce gastric secretion, except by reflexly increasing an existing vagus secretory tonus. Moreover, the presence in the stomach of a rubber tube or other collecting devices is necessary even for observations on the empty stomach of normal men and experimental animals.

Rehfuss, Bergheim and Hawk have reported many cases in which the gastric secretion continued for a half to one and a half hours after all the food (Ewald meal) had left the stomach. This secretion might be

explained on the basis of a slow absorption of gastrin bodies produced by the preceding meal or as due to intestinal reflexes acting on the gastric glands. These factors should be eliminated when the observations are made in the morning before breakfast, that is, 12 hours after the meal. When this is done in normal persons, using the Rehfuss tube, we practically never fail to demonstrate a continuous gastric secretion of relatively low acidity, even though the observation be continued for hours. The quantity is from 30 to 60 cc. per hour. The admixture with swallowed saliva is difficult to control; duodenal regurgitation is also a disturbing factor. But the continuous gastric secretion is so universally present in normal persons that controls must be run on this factor in all accurate studies of gastric secretion.

The continuous gastric secretion is usually also in evidence in dogs with a Pavlov stomach pouch. This is denied by Bickel and Rheinboldt, but it has been demonstrated in our laboratory in the several lines of study of gastric secretory factors in such animals, particularly if the animals are in good condition. At times the secretion appears to be mainly mucus and pepsin, free HCl being absent.

Bickel has demonstrated a continuous gastric secretion in the goat. This will probably be found true for all the ruminants. Schalk studied the secretion of a Heidenhain pouch of the true stomach of the goat. The secretion was continuous even in prolonged absence of food. Three 72-hour fasting periods were run. In the first period 17 one-hour records were taken with an average secretion rate of 7 cc. In the second period 25 one-hour records showed an average secretion rate of 8.5 cc. At the end of the third fasting period the secretion rate was down to 3.5 cc. per hour, but equally low rates were sometimes noted on days when the goat had a full supply of food. The acid and pepsin of the gastric juice on the fasting days showed no deviation from the normal.

It is clear, however, that accurate studies of secretory factors even in Pavlov pouch dogs must include controls on the continuous secretion. The term "continuous" should not be misunderstood in this connection. The secretion is usually not uniform in rate but the periodicity of the continuous secretion is less marked than the periodicity of the motor phenomena in the empty stomach.

C. The continuous gastric secretion in prolonged fasting. Pavlov reported that the secretion of gastric juice on sham feeding in a prolonged fast ceases after eight or nine days unless NaCl is given in the drinking water. If the NaCl is sufficient the fasting itself is reported as not materially influencing the quantity or the quality of the appetite gastric

juice. Boldyreff, using dogs with gastric and intestinal fistulae, found that after 24 hours of fasting the gastric glands begin to secrete an abundance of juice periodically, and after 3 to 4 days of fasting the gastric secretion becomes continuous and so abundant as to inhibit the motility of the fasting stomach. Carlson found in man, fasting for 5, 8 and 15 days, no significant change in the rate of continuous secretion.

The acidity of the continuous secretion showed on the whole a slight increase with the duration of the fast, and with this appeared a greater frequency of regurgitation of intestinal content into the stomach. This continuous secretion is greatly augmented over a long period on breaking the fast. This has recently been confirmed by Kunde on Pavlov pouch dogs. Sutherland studied the continuous secretion in fasting dogs provided with Pavlov and Heidenhain stomach pouches. He reports persistence of the secretion, with some periodic variations in the secretion rate throughout the fast. He found a gradual decrease in the quantity of the secretion unless NaCl was given with the drinking water. Hess and Taylor have reported a continuous secretion of gastric juice in the newborn infant before ingestion of food. Taylor gives the quantity as 60 to 200 cc. in 24 hours. In the guinea pig there may be demonstrated a continuous secretion of gastric juice during late intrauterine life (Sutherland).

Politzer reports marked secretion of gastric juice before the first feeding in 100 human infants. He suggests that swallowed amniotic fluid may be the stimulus to this secretion.

The mechanism or the cause of the continuous gastric secretion is unknown. It is probably not appetite or psychic secretion, as this term is ordinarily understood. It may be a subconscious secretory tonus of the vagi, in which case the secretion should cease on double vagotomy. But this is not the case. We may have secretogogues produced in the autodigestion of the gastric juice itself, as well as by the digestive and bacterial processes in the intestines. Jarno and Hik think that the secretion follows periods of gastric hunger contractions. It is well known, however, that the secretion may persist during the gastric hunger contraction period as well as during the period of motor quiescence.

Luckhardt and Johnstone have recently found that the continuous gastric secretion in man is augmented on the induction of hypnotic sleep. A similar augmentation will probably be found to occur in normal sleep. This seems to indicate an inhibitory tonus (via the vagi?) on the gastric glands, similar to that found by Carlson for the gastric motor mechanism. These recent observations of Luckhardt and Johnstone

seem to demand a re-investigation of inhibitory secretory nerves to the gastric gland, the existence of which Pavlov thought he had demonstrated.

D. The appetite secretion of gastric juice. 1. Active secretion of gastric juice in the dog on seeing food was recorded by Bidder and Schmidt in 1852, and in 1878 Richet observed gastric secretion in man on tasting various foods. But it remained for Pavlov and his pupils to work out in greater detail this secretory mechanism by his classical experiments on dogs. Subsequent to Pavlov's researches, a number of investigators have worked on men with gastric fistulae and stenosis of the esophagus, conditions that closely parallel Pavlov's experimental animals (Kaznelson, Hornborg, Umber, Bogen, Sick, Carlson, etc.). The following points seem definitely established for man, monkeys, dogs and cats.

1. Seeing, smelling and tasting of food induce gastric secretion, provided the state of hunger and appetite is present. It is therefore a conditioned reflex (Bogen, Bickel). Tasting the food appears to be the most potent stimulus in most individuals; seeing and smelling the food may be without effect in some persons.

2. The vagi nerves constitute the sole efferent paths of this reflex. But direct stimulation of the vagi nerves yields little or no secretion of gastric juice, possibly because of the presence of nerve fibers that inhibit the gastric secretion (Pavlov).

3. The mere act of chewing indifferent substances and the stimulation of the nerve endings in the mouth by substances other than those directly related to food, causes no secretion of gastric juice.

4. Sham drinking of water on the part of the thirsty animal starts secretion of gastric juice (Carlson, Orr and Brinkman). This may be be due to a close association of the cerebral processes of hunger and thirst. Thirst may also have an inhibitory action on gastric secretion due to the concentration of the blood, as well as to the subjective processes of the feeling of discomfort.

5. Appetite secretion of gastric juice seems to be absent in adult ruminants (Bickel). If this is true it is probably a secondary adaptation or loss during the growth of the individual. It would seem that the mechanism would be as essential or useful to the sucking calf as to the sucking infant. Appetite secretion of gastric juice in the adult ruminants may be induced by the second mastication only.

2. The latent period of the appetite secretion is about 4 to 5 minutes in the dog (Pavlov). In man the latent period is shorter, especially if

the gastric glands are in a state of relatively active continuous secretion (Carlson). In the dog after 24 hours' fast, sham feeding for 5 minutes may initiate gastric secretion lasting for 4 to 6 hours (Pavlov, Rosemann). In man the appetite secretion is more transitory, usually ceasing within 15 to 20 minutes after completion of mastication (Carlson).

3. The rate of the appetite gastric secretion varies directly with the palatability of the food, and the degree of hunger and appetite. The maximum secretion rate in large dogs is reported as 4 to 8 cc. per minute (Konowaloff, Rosemann). In an adult man Carlson found the appetite gastric juice secretion rate to be on the average 3.5 cc. per minute, the lowest being 1.5 cc. per minute, the highest 11 cc. per minute. There is probably a great variation in this secretion rate even among normal individuals.

The quality of the appetite gastric juice depends on the secretion rate, and is independent of the character of the food.

4. We have several reports on the secretion of gastric juice in man induced by hypnotic suggestion. This phenomenon involves, of course, the nervous secretory mechanism. Bennet and Venable (using the Rehfuss tube in hypnotized subjects) found that suggesting hunger, food and eating caused secretion of gastric juice, while suggesting nausea depresses the gastric secretion. Heyer aspirates the gastric juice in hypnotized persons by means of a Nélaton catheter, and reports that on suggesting the eating of a meal there is a marked increase of the gastric secretion. This is a kind of sham feeding. Heyer reports also that suggesting pleasant or unpleasant experiences depresses this gastric secretion. This depression is most marked with the unpleasant suggestion.

Luckhardt and Johnstone, in our laboratory, have found that suggesting the eating of the meal to suitable hypnotized subjects produces as copious gastric secretion as the mastication (and spitting out) of a similar meal when the person is awake. The gastric secretion induced by the suggestion of eating in the state of hypnosis is very transitory. Needless to say that in these experiments the continuous secretion factor was carefully controlled.

In this work Luckhardt and Johnstone discovered a fact that seems to be of fundamental importance in regard to the nature of the nervous control of the gastric glands. They found that the continuous gastric secretion is invariably increased by the state of hypnosis itself, and in the absence of all suggestions of food. This fact points to an inhibitory

nervous tonus acting on the gastric glands in the waking state, unless the inhibition should be central (central depression of the vagi secretory fibers). It is probable that normal sleep likewise increases gastric secretion, just as normal sleep increases the tonus and contractions of the stomach. According to Tomaszewski, there is a paralytic secretion or hypersecretion in the Heidenhain gastric pouch for the first 7 to 10 days. It will be recalled that the gastric vagi fibers to this pouch are severed. Bickel and Rheinboldt also describe a continuous secretion in the Heidenhain pouch but not in the Pavlov pouch. These new facts give an additional incentive for renewed investigation of the mechanisms of inhibition of the gastric glands.

5. The significance of the appetite secretory mechanism in normal digestion is not clear. Pavlov identifies it with appetite and argues that both appetite and gastric secretion are necessary for the initiation of normal digestion. Some of his experiments on dogs seem to support this prevalent view. However, double vagotomy in dogs does not seriously impair digestion, administration of alkali in man to the point of complete neutralization of the gastric juice acidity does not seem to impair digestion, and all the appetite secretion induced by the normal mastication of a meal in man may be removed from the stomach before admitting the food (via gastrostomy) without impairing digestion or delaying the emptying of the stomach (Carlson). If the mechanism is not necessary for health, it is at least a factor of safety. But the continuous gastric secretion is sufficient to initiate gastric digestion. This new aspect of the appetite gastric secretion minimizes the importance of therapeutic measures that are supposed to induce or augment the appetite gastric secretion.

E. Appetite gastric secretion in the newborn. Practically all observers agree that at birth the gastric mucosa of mammals is sufficiently differentiated to secrete normal gastric juice, except possibly for a slightly lower acidity and ferment concentration. The prematurely born infant secretes gastric juice (Politzer). In dogs and cats the gastric glands respond to gastrin when the fetus is within a few days of term, and in the guinea pig there may be spontaneous secretion of gastric juice in utero (Sutherland). But the question whether in the newborn mammal the appetite gastric secretory mechanism is already perfected is still open. Cohnheim and Soetbeer report secretion of gastric juice in newborn pups on sham feeding and, on nursing dry breasts. Noetemann reported appetite gastric secretion in a $3\frac{1}{2}$-year old child with gastric fistula and stenosed esophagus. Bogen's negative results might

have been due to so-called psychic inhibition. Taylor's work on numerous human infants seems carefully controlled, and he concludes that the appetite gastric secretory mechanism is not developed or functional in the newborn. If this is correct there remains to be determined at what age this mechanism becomes functional in man, and work should be done to determine the first appearance of the mechanism in the lower animals. The appetite secretion mechanism in the newborn ruminant should be investigated, in view of the report that it is not functional in adult ruminants (Bickel).

F. THE SECRETION OF GASTRIC JUICE INDUCED BY FOOD IN THE STOMACH AND INTESTINES. *1. Direct mechanical factors.* Supported by Beaumont's experiments on Alexis St. Martin, the view that the mechanical stimulation of the gastric mucosa by foods induced secretion of gastric juice was generally accepted. Pavlov's experiments on dogs seem, indeed, convincing enough in eliminating this mechanical factor. But I do not believe that the matter is definitely settled. To be sure, in man and most of the higher animals the cells that secrete the gastric juice (apart from the gastric mucin) are situated so far away from the main cavity of the stomach that mechanical contact of the solid food particles with these cells is out of the question. But this does not exclude the possibility of the food acting mechanically through reflex mechanism. This possibility is not even excluded by the fact that the Heidenhain and the Pavlov accessory stomachs secrete gastric juice when food is present in the main stomach, for it is very difficult to sever all nerve connections with these stomach pouches without interfering with the blood supply. Such mechanical stimuli as sand blasts, test tube brushes or glass rods applied to the gastric mucosa may not be adequate. They may, indeed, be violent enough to induce reflex inhibition of the gastric secretion.

Pavlov has advanced the view that mechanical stimulation by the food of the sensory nerve endings in the gastric mucosa may induce the conscious sensation of appetite and thus through a central reflex induce gastric secretion. Some experimental evidence is cited in its support, but the view is at the most a working hypothesis, with most of the work yet to be done.

2. The action of water. Heidenhain, and Pavlov and his pupils showed that water in the stomach excites the secretion of gastric juice under experimental conditions that seem to exclude the psychic or appetite mechanism. The fact itself has been confirmed both on man and experimental animals by a number of subsequent investigators.

The literature is reviewed by Ivy and Sutherland. These authors, working in our laboratory, have endeavored to determine the mechanism of this water stimulation. Sutherland found that water and salt solutions (iso-, hypo- and hypertonic) stimulate the gastric glands. Water introduced directly into the intestines stimulates gastric secretion but to a less extent than does the same quantity of water in the stomach. Water given per rectum has little or no effect on the gastric glands, but Heyer has recently reported that distention of the rectum by enemas causes a reflex secretion of gastric juice. This needs confirmation and analysis. Food in the alimentary canal increases the gastric response to water introduced in the stomach or intestines. Ivy found that there is great variation in the gastric response to water in different individuals, and that on the whole the longer the water remains in the stomach the greater the gastric secretion.

Walenko reports that intravenous injections of hypertonic salt solutions induce secretion in the Pavlov pouch, and depresses the secretion in the Heidenhain pouch, explaining the latter by direct osmosis, the former by osmotic stimulation of the nervous secretory mechanism.

The response of the gastric glands to intravenous injections of water and salt solutions is probably an instance of the general tissue action in controlling hydremias. It seems also probable that water in the stomach and intestines may augment gastric secretion by a more rapid absorption of secretogogues.

3. The peripheral action of proteins, carbohydrates and fat. Under experimental conditions excluding the appetite secretion, so far as this is possible, as well as the entrance of the foodstuffs into the intestines, Pavlov and his pupils reported that meat extracts and fatty acids induced gastric secretion after a long latent period (15 to 30 minutes). Proteins in bread, raw or coagulated egg white, and meats extracted by boiling caused practically no gastric secretion, a slight secretion appearing after a latent period of more than an hour, and explained by Pavlov as due to secretogogues developed in the course of the digestion of these substances. Pure carbohydrates induced no gastric secretion. Fats by themselves had no effect, but given with meat or meat extracts the fats depressed both the appetite and the local or "hormone" secretion. Fatty acids stimulate the gastric gland, but Pavlov stated that hydrochloric acid itself tends to inhibit the gastric glands, and he sees in this a kind of autoregulative mechanism of the secretion.

According to Pavlov and his pupils meat, meat extracts or digested egg white have very little effect when introduced into the duodenum,

and no effect when introduced into the large bowel or parenterally. The fats inhibit gastric secretion even when introduced into the duodenum but this inhibition is decreased by section of the vagi (Orbeli). These investigators also report that bile and duodenal secretion, introduced into the stomachs stimulate the gastric glands.

These facts, based on experiments on dogs, have in the main been confirmed by subsequent observers both on man and experimental animals. The clinical literature is conflicting in regard to the effects on the gastric secretion of nutrient enemata, the discrepancies being probably due to failure to control the continuous and the appetite secretion, and the uncertainties of the ordinary clinical examination of gastric content. The main effort in the field in recent years has been attempts to work out the mechanism of this apparent local action of some of the food substances on the gastric glands.

Pavlov took the view that it was a reflex mechanism, there being efferent nerve endings of specific sensibility in the gastric mucosa. Popielski showed that we are not dealing with long reflexes, because the phenomenon persists after isolation of the stomach from the central nervous system. On the basis of this fact Popielski proposed the theory of local reflex mechanisms. This theory has so far neither been proved nor disproved, but it is at present relegated to the background by the *gastrin theory*. This theory was initiated by the work of Edkins, who thought he had demonstrated a specific gastric secretogogue in the pyloric mucosa. The gastrin theory postulates that substances either in the native foods or developed in the gastric digestion of foods, act on the pyloric mucosa in such ways that a gastric secretogogue is produced, this is in turn absorbed into the blood and acts on the fundic glands via the blood.

Now, what are facts? In the first place, the alleged fact that meat extracts, or the end products of gastric digestion of proteins fail to cause gastric secretion when administered parenterally seems to support the theory to the extent that it eliminates the possibilities of secretogogues or gastrins in the food itself. We said "alleged facts," because more recent work has shown that gastric secretogogues or gastrins can be obtained from a variety of plant and animal tissues by hydrochloric acid extraction. The same chemical procedure has shown their presence in the mixed foods of the ordinary meal. Frouin secured gastric secretion both on ingestion and on hypodermic injection of gastric juice. Kisseloff reports gastric secretion on intravenous injection of watery extracts of strawberry, lettuce and spinach. Eisenhardt secured secre-

tion in the denervated Pavlov pouch on subcutaneous injection of extracts of meat, digested bread, extract of spinach and normal (entire stomach) gastric juice. Injection of fundic juice gave no secretion.

But on the positive side of the theory it has been shown that Edkins' experimental methods were so faulty that nothing can be concluded from his results (Ivy). The work of Gross supports the gastrin theory in so far as he found that meat extract caused gastric secretion when brought in contact with the pyloric mucosa. Gross' findings are directly contradicted by Ivy, who used better experimental methods. Ivy isolated the antrum pylori in Pavlov pouch dogs, and united the stomach with the duodenum. These dogs remained in good health over long periods. Ivy reported that substances like gastric juice, $\frac{N}{10}$ HCl, Koch's gastrin, dextrose, peptone, fresh meat extract and Liebig's meat extract when kept in contact with the pyloric mucosa for an hour or more, have no effect on the gastric (fundic) glands. This would be an *experimentum crucis*, completely overthrowing the gastrin theory in its present form, provided we could feel certain that the pyloric mucosa was normal. Ivy showed that some of the pyloric mucosa functions (e.g., secretions, absorption) remained normal.

The work of Popielski, Ehrmann, Ensman, Eisenhardt, Maydell, Tomaszewski, Kissiloff, Koch, Luckhardt, Keeton, Rogers and Fawcett and others has demonstrated that a gastric secretogogue can by suitable means be secured not only from the pyloric mucosa but from the mucosa of the entire alimentary tract, and from such organs as the liver, the thyroid, plant tissues, etc. This has recently been denied by Lim, on basis of acute experiments on dogs under chloroform anesthesia. Negative results on anesthetized dogs cannot be used to contradict positive results on Pavlov pouch dogs.

Luckhardt has recently shown that gastrin (or the gastrins) is not specific for the gastric gland, since it stimulates both the stomach and the pancreas. The pancreatic secretin of Bayliss and Starling likewise stimulates both the pancreas and the stomach. It seems highly probable from present data that the gastrins are artefacts developed in the decomposition of the foods, or in the extraction of the mucosa and do not represent physiological mechanisms. They are all without action when given by mouth.

The action of the gastrin on the stomach is decreased but not completely stopped by atropin. We have thus an antagonistic action similar to that between pilocarpin and atropin. The gastrins probably belong to the pharmacology rather than to the physiology of gastric function.

The gastrins act more effectively on hypodermic and intramuscular than on intravenous injection. There is some evidence that the gastrins are rapidly destroyed by the liver (Bickel, Djenah).

The chemistry of the gastrins. Popielski endeavored to prove that the gastrins (and secretins) were identical with the vasodilatins, the gland activity being a secondary effect of the capillary dilatation. This view has neither been conclusively proved nor disproved. But Luckhardt has recently shown that vasodilator substances do not necessarily initiate gland action. Histamine is a general secretogogue, and Popielski has advanced the view that this is the active substance in organ extracts. The studies of Koch, Luckhardt and Keeton indicate that gastrin is an imidazol derivative, like histamine and pilocarpin. They were able to separate histamine and gastrin by chemical means, which seems to show that they are different substances.

The gastrin theory may be correct but all the evidence on which it is based appears to have been completely disproved. If the theory is correct, the real gastrin has so far eluded direct detection and the whole subject of how the foods in the gut cause secretion of gastric juice must be re-investigated. The possible reflex factors have been neglected in recent years. The inhibition of the appetite secretion in the Pavlov pouch by fats in the stomach or in the intestine can hardly be explained on any other basis than as a reflex inhibition. And if we have inhibitory nervous reflexes from wide areas of the gastro-intestinal mucosa to the fundic glands, it is not probable that excitatory reflexes are absent. Tomaszewski thinks the secretogogues of meat (gastrins) act on local reflex mechanisms or directly on the gland cells. The latter possibility is eliminated by the fact that all the gastrins are without action when given by mouth.

Gastric secretion from foods in the small intestines. According to Pavlov and his pupils, the foods that have a marked stimulating action on the gastric glands when in the stomach have little or no action when introduced into the intestines. These results have been contradicted, at least in part, by later investigators. Tomaszewski states introduction of peptones into the intestines causes secretion of gastric juice if the vagi are intact.

Ivy has recently studied this question with improved technique. The dogs are provided with a Pavlov pouch and a fistula of the duodenum, the jejunum being sutured to the pyloric end of the stomach. These dogs remain in good condition. Meat extract, gastric juice and hydrochloric acid induce secretion of gastric juice after a latent period

of 30 to 45 minutes. Digested meat and Leibig's meat extract are without effect. Butyric acid, alcohol, glycerine, soaps and spinach extract in the duodenum likewise induce gastric secretion. Glucose, olive oil and mustard oil are ineffective. Gastric secretion is induced by NaCl (10 per cent) but not by Na_2CO_3.

The secretion of the glands in the pyloric antrum. The gastric secretion discussed in the foregoing section is that of the fundus or cardiac end of the stomach. In the mammals (excepting the ruminants) the pyloric mucosa secretes neither HCl nor pepsin. The pyloric secretion is alkaline, and is not increased by the various foods and secretogogue factors that stimulate the fundic glands. The literature is reviewed by Ivy (1921).

4. The influence of acids, alkalis, salts, food condiments, alcohol and bitter "tonics" on the gastric secretion. (1) We have seen that acids (HCl, fatty acids, normal gastric juice) appear to induce gastric secretion acting both from the stomach and the small intestines. The action of alcohol seems also clear as to the fact itself in that, however administered (per os, per rectum or parenterally) it excites the gastric gland (Chittenden et al., Chiari, Zitowitsch, Kast). The mechanism of this action is not definitely worked out. It may be, in part, a central depression of the inhibitory tonus governing the gastric glands in the normal animal. If this is the case alcohol should have much less action on the Heidenhain pouch as compared to the action on the Pavlov pouch. Ehrmann's observations do not support this view.

(2) The present information on the influence of acids in the gut on the gastric glands is both scanty and conflicting. Ivy obtained no effect from acids in the antrum pylori, but acids in the duodenum caused some secretion in the stomach after a long latent period. Pavlov claimed that acids in the stomach tend to inhibit gastric secretion, and Carlson found in man that frequent removal of the continuous secretion yields a greater total secretion per hour than hourly aspirations. Pavlov's view seems to be contradicted by the copious secretion of gastric juice in pyloric obstruction in man.

(3) Pavlov and his pupil Chigin state that NaCl has little or no influence, while Na_2CO_3 in the stomach depresses gastric secretion. Subsequent work in this field, mainly with the alkalis, has yielded very discordant results. Lönnquist, Rosenblatt, and Bickel found that NaCl stimulated gastric secretion. Most of the observers (Rosenblatt, Ehrmann, Chiari, Hirsheimer, Bickel) report a depression of gastric secretion by the $NaHCO_3$. Others class the $NaHCO_3$ as a gastric stimulant.

Most of these observations were made on dogs with accessory gastric pouches. Observations on man using the fractional analysis have not yielded conclusive results (Crohn). Boyd, working on Pavlov pouch dogs in the author's laboratory, found that $CaCO_3$ and $NaHCO_3$ usually stimulate gastric secretion until the quantity of alkaline administered (1.5 gr. or more per kilo body in 2 hours) induces nausea and vomiting. This depresses the secretion. This dose corresponds to about 100 grams $NaHCO_3$ given per os to an average adult person in the course of 2 hours. Boyd's experiments were carefully controlled.

The practical interest in the action of alkalis on gastric secretion lies in the extensive use of carbonated drinks, and in their extensive clinical use to control gastric acidity and hypersecretion, especially in gastric and duodenal ulcers. It would seem that we have been using gastric secretogogues to control and depress gastric secretion. Of course, if enough of the alkalis are given, and if they remain long enough in the stomach (there are reports in the literature to the effect that alkalis hasten gastric evacuation) the direct neutralization may more than counteract the secretogogue effects.

The mechanism of the salt and alkali action on the gastric gland activity has not been satisfactorily worked out, despite its practical importance. Ehrmann states that $NaHCO_3$ and NaCl have no inhibitory action on the Heidenhain pouch, similar to that on the Pavlov pouch. If this is correct, it indicates a reflex action from the stomach of these chemicals. He also reports that fats, soaps, morphine and concentrated sugar solutions have no influence on the Heidenhain pouch secretion; hence the action of these substances on the Pavlov pouch must also be a reflex.

It is difficult to account for these discordant results except on the basis of defective control of such factors as the appetite and continuous secretion, water intake, thirst, and the physiological state of the experimental pouch. Salts or alkalis will, of course, change the concentration of the blood, influence thirst, and therefore the water intake. Sutherland found that intravenous injection of iso-, hypo- and hypertonic NaCl solutions stimulates the gastric glands. This is probably in part an effect of the plethora.

Since the alkalis alter the control of the pylorus to a certain extent, this factor must also be controlled. The absorbable alkalis may influence the availability of the chlorides in the gastric glands, and thus influence the HCl secretion, but this does not seem a probable factor, since the animal must be in chloride starvation for a long time before the HCl of the gastric juice is appreciably affected.

It is stated by some clinicians (personal communication) that administration of large doses of alkalis over a long period (weeks) may produce an achylia lasting for several weeks after discontinuing the alkalis. This is important, if true, and would indicate a direct injury to the gastric glands. This condition has not been produced under adequate experimental control.

(4) Coffee, cocoa (minus most of the fat) and chicory in the stomach are reported to stimulate gastric secretion. Tea has no effect or may act as a depressant. The alkaloid caffeine has a slight stimulating action both on the Heidenhain and the Pavlov pouch (Pincussohn, Bickel, Ehrmann). Coffee, tea and cocoa are watery extracts of plant tissues, and as such may contain the gastrin bodies. But this direct action of these beverages on gastric secretion is of little practical significance, since the water content and the taste are greater factors in augmenting the gastric secretion.

The common food condiments acting from the stomach have little or no effect on the gastric secretion (Rabinowitch). The essential action of these substances is on the appetite secretion through taste. Raw onions, acting in the stomach, are reported to have a more pronounced secretogogue action than most vegetables (Wilbrand).

(5) Various bitter substances or bitter tonics have been used in medicine from time immemorial to aid gastric digestion. Do these subsubstances increase the secretion of gastric juice? Pavlov and Borissow report that bitters given before the meal increase the appetite gastric secretion by increasing the excitability of the nerve endings in the mouth. Reichmann and Scheffer found that bitters in the stomach depress gastric secretion. Kaznelson and Bickel report that quinine in the mouth stimulates gastric secretion in man, but their experiments were not adequately controlled.

Carlson and Moorhead found that bitters had no appreciable effect on gastric secretion in normal man and normal dogs. In dogs rendered cachectic (experimental anemia), the bitters seem to have a slight stimulating effect on the gastric secretion, but this is too insignificant to have any real therapeutic significance. The value of bitters, if any, in human dietetics must be sought in other factors than in the augmentation of gastric secretion. Moorhead found that bitters given to cachectic patients may slightly increase the intake of food.

(6) Direct stimulation of the gastric gland with the direct or induced electrical current does not cause secretion of gastric juice. The x-ray and radium are also reported to be without effect, except for chronic

depression of the gastric gland when massive doses are used (Brenzel, Winternitz, Szegö).

(7) The secretion of the gastric mucin is a process largely independent of the secretion of water, HCl and ferments. All irritants, including mechanical and electrical stimulation, increase the mucin secretion, without increasing secretion of gastric juice.

5. The reflex versus the hormone theories of the local gastric secretory mechanism. (1) We have seen that the hormone or gastrin theory has not been disproved, but most of the data supporting it have been shown to be due to errors in technique or interpretation. What about the theory of local reflex mechanism as originally advanced by Pavlov? Some of the differences in the secretory response of the partially denervated Heidenhain pouch and the Pavlov pouch, which retains part of its vagi innervation, may be explained by absence of the secretory reflexes through the central nervous system, and by gradual deterioration of the glands in the denervated pouch. But I am not convinced that these are adequate explanations for all differences, despite the modern tendency to deny the existence of all local reflex mechanisms in the viscera. Many of the contradictory results are probably due to failure to control the continuous secretion and the vasomotor factors. The latter factor will probably influence the former. Unless the augmentation of the secretion in the Heidenhain pouch by the presence of food in the stomach and intestines can be explained on the basis of augmentation of the continuous secretion through circulatory changes, this would seem a strong support for the hormone or gastrin theory. Such secretion responses seem to obtain even after severance of the continuity of the Auerbach and the Meissner plexuses, but vasomotor reflexes (and possibly secretory reflexes) may go on via the nerve fibers along the blood vessels. Rheinboldt reports that the result of section of the nerves along the blood vessels to the Heidenhain pouch reveals some secretory function of those nerves, but his data are not conclusive.

(2) In normal men and animals all painful stimuli cause some inhibition of the entire phase of gastric secretion. Pain in certain pathological states in man (e.g., gastric and duodenal ulcer pains, pains in intestinal obstruction, etc.) seem to constitute an exception to this rule. Depressant emotions (fear, anger, anxiety, etc.) also depress both the appetite secretion and the local secretion phase. Strong emotions of pleasure seem also to cause some depression of the gastric secretion. Hence gastric secretion may be depressed from changes in the brain, from painful stimuli to the skin, and from certain types of stimuli (e.g., fat)

to the mucosa of the alimentary tract. The relative rôle played in this inhibition by stimulation of inhibitory secretory nerves, vascular changes in the stomach, changes in gut motility, changes in gastrin absorption, and changes in the activity of such remote organs as the adrenals and the thyroid, remains to be worked out.

The action of drugs on gastric secretion, such as the stimulation by pilocarpin and nicotin (Ehrmann, Skaller) and the depression by atropin (Keeton, Luckhardt and Koch) indicate but do not prove the preponderance of the nervous mechanisms. It seems significant, however, that atropin will completely inhibit the secretion from food in the stomach and intestines, while it decreases but does not completely inhibit the secretion from histamine and the gastrins. Adrenalin and pituitrin depress gastric secretion (Hess and Gundlach, Pal). This may be a vasomotor factor.

6. Quantitative and qualitative adaptation of gastric secretion to the food. (1) The appetite gastric juice is only quantitatively related to the food, in that, other things being constant, the more appetizing the food the more copious the secretion. This is true even of foods that do not require digestion or cannot be digested in the stomach. Arrhenius has attempted to show that there is a mathematical relation between the quantity of the ingesta and the quantity of the gastric juice produced. But the most important contribution to this subject has come from Pavlov's laboratory (Chigin). Thus Pavlov distinguishes between "meat juice," "bread juice" and "milk juice" as follows:

	SECRETION PERIOD	SECRETION TOTAL	ACID CONCENTRATION	PEPSIN CONCENTRATION
Meat	Medium	High	High	Medium
Milk	Short	Low	Medium	Low
Bread	Long	Medium	Low	High

According to Pavlov the stomach secretes more than double the quantity of pepsin for bread proteins than it does on equal quantities of meat or milk protein. These apparent qualitative adaptations of the gastric juice to the foods are given teleological interpretation by Pavlov. It is argued, for example, that the low acidity and high pepsin concentration of the "bread juice" is advantageous for the digestion of vegetable proteins.

Assuming that the above facts are as stated (Arloing, Cade and Bocca) they seem to demand *a*, a relative independence of the processes of water, HCl and pepsin production by the gastric glands; *b*, specific

sensibilities of the afferent path in the reflex secretory mechanisms in the gastric and intestinal mucosa as assumed by Pavlov; or *c*, different kinds of gastrins (secretogogues) in the food acting with different intensities on the three secretory processes. In some animals at least the HCl and the pepsin appear to be secreted by different gland cells, and there is other evidence that the secretion of HCl and pepsin are more or less independent processes, but the other necessary elements of the theory (specific sensibilities of the reflex mechanism, or specific gastrins for each food) are as yet imagined entities.

The fact itself (the qualitative adaptation) should be reinvestigated in light of the more recent work on the gastric glands, and gastrointestinal motility as influenced by the character of the food. In view of the variations in the gastric response to the same meal from day to day in the same animal, the original data of Chigin do not appear to me conclusive; and there is a seeming discrepancy between the rapidity with which a predominantly carbohydrate meal like bread leaves the stomach (Cannon) and the prolonged duration of the gastric secretory response to bread. There is some evidence that the acidity of the gastric juice increases with the secretion rate, while the pepsin concentration may actually decrease with the secretion rate (Carlson). This factor alone may explain the qualitative difference between the "meat juice" and the "bread juice" of Chigin.

7. *The composition of normal gastric juice.* (1) We have many analyses of pure gastric juice of the dog (appetite and digestion juice) and man (appetite juice), the latter secured from gastric fistula cases. The data from various observers are in essential agreement, and there appears to be little or no difference in composition of pure gastric juice in dog (Rosemann) and man (Carlson). Normal human gastric juice has the following composition:

Acidity { Free HCl = 0.40–0.50 per cent
Total acidity = 0.45–60 per cent
Solids { Organic = 0.42–0.46 per cent
Inorganic = 0.13–0.14 per cent
Specific gravity = 1006–1009
Osmotic concentration = −0.55°–0.52°C.
Total nitrogen = 0.051–0.075 per cent
Amino acid nitrogen = 3–10 mgm. per 100 cc.
Ammonia = 2–8 mgm. per 100 cc.
Chlorides = 0.50–0.58 per cent

The concentration of the ferments and the mucin cannot at present be expressed in percentages.

Bacteria are present in normal gastric juice both in man and dog. The antiseptic action of gastric juice has been known since the days of Spallanzani, but the bactericidal power of gastric juice is generally over-estimated. Burget found 25,000 to 100,000 organisms (yeast, bacilli, cocci) per cubic centimeter of normal human gastric juice (full normal acidity and peptic power). Poppens found B. coli, staphylococci and non-hemolytic streptococci in the gastric juice of dogs.

(2) The gastric juice secreted at a rapid rate has a higher acidity than that secreted at a slow rate. This is probably due in part to neutralization by gastric mucin, and formation of ammonia from the protein of the gastric juice, when saliva and regurgitated duodenal juice are excluded. This is indicated by the fact that the total chlorine of the slowly secreted juice is not as low as would be indicated by the low acidity. But there appears also to be some actual increase in acidity with the increase in the secretion rate (Carlson, Rosemann).

The evidence in favor of the view that the HCl is produced by the "border cells" of the gastric glands seems fairly conclusive, but we do not know how or where these cells produce it. In fact, there is some evidence that the acid may not be produced as such within or even on the surface of the cells. Using the Prussian blue reaction originally tried by Claude Bernard, Fitzgerald reported that the HCl was produced within the parietal cells. After showing the unreliability of the Prussian blue reaction, Harvey and Bensley concluded, on the basis of a different stain reaction, that the HCl first appeared in the lumen or neck of the gland at considerable distance from the parietal cells, while Collip appears to have proved that the HCl is formed on the cell border. The discrepancies in the results obtained so far by the staining methods are probably in part due to the condition of the gland cells when the stain is applied and the cells actually seen. From what we know of gastric secretory activity, it seems highly probable the cells observed by these investigators were not actively secreting, but were dead or moribund. Experiments bearing on this question are not physiological, unless the cells are secreting gastric juice when observed, or unless the chemicals used in the reaction are known not to impair the secretion process at least before they reach the interior of the cells. Unfortunately, the smaller transparent invertebrates, where one can observe the gastric mucosa under the microscope in the living animal, do not secrete an acid gastric juice. But it is possible that sulphuric acid secreting salivary glands in some molluscs is a better object than the veterbrate stomach for the solution of the problem how living cells produce in-

organic acids in relatively strong concentration. The high concentration of the HCl in the gastric juice has been cited as an argument against the production of the acid within the cells, as such high concentration of of a strong acid would kill the cell itself. This point does not seem well taken. The gastric juice in contact with the surface of *living cells* does not kill all cells. This is true not only for the mucosa of the alimentary tract, but for organs not "acclimatized" to gastric juice, like the spleen and the kidney (Dragstedt).

The secretion of HCl in the gastric juice is not intimately dependent on the chlorides of the food, because complete chloride starvation must be maintained such a long period before the HCl of the gastric juice disappears (Wohlgemuth, Rheinboldt, Takata, Rosemann). In chloride starvation the gastric glands can apparently produce HCl until the secretion itself ceases from general cachexia.

The theory of Harvey and Bensley that parietal cells secrete the HCl as an organic or colloid compound demands investigation, if it should be shown that the acid is not liberated in or on the surface of the cells. Rosemann has suggested an actual secretion of lactic and phosphoric acids by the gastric glands under certain conditions.

(3) The origin and possible significance of ammonia of the gastric juice has recently been investigated by Huber on man and dogs. He found marked but consistent individual variations in apparently healthy subjects. The gastric juice ammonia is increased by a high protein diet and by adding ammonium salts to the food. This indicates an excretion from the blood. The fundic mucosa contains more NH_3 than either the pyloric or the cardiac mucosa. This may indicate a relation of the gastric juice ammonia to the hydrochloric acid secretion. The bacteria of the gastric juice may also be a factor. The gastric juice ammonia appears to have no pathological significance, as the concentration may be as high in normal individuals as in patients with gastric ulcer and gastric cancer.

(4) The most important ferments of the gastric juice are the pepsins. During relative quiescence of the gastric glands the pepsins in an inactive form (pepsinogens) are stored up, possibly in the form of granules, in the chief cells. The pepsinogens are activated by acids. This may be one significance of the differentiation of HCl and pepsinogen production in separate cells. Lipase is also present in pure gastric juice (Davidsohn, Hull and Keeton). This lipase appears to be an actual secretion of the gastric gland rather than a diffusion product from the blood and lymph, as it appears to be present in much higher concentration in

the gastric juice. The physiological significance of the gastric lipase is obscure, as it is quickly destroyed by the acidity of normal gastric juice.

The long controversy as to the identity of the pepsin and the rennins seems to have ended in favor of Hammarsten's view that they are distinct groups (Taylor, Levy, Burge, Hammarsten). If this is correct the biological significance of the rennins becomes a riddle, as they are present in the gastric secretion from the fish up, that is, in animals where milk is absent from the diet.

IV. THE SECRETION OF GASTRIC JUICE IN DISEASE. The literature on the pathological physiology of gastric secretion is very voluminous, also very conflicting, not so much in the facts reported as in the significance in the disease complex assigned to the altered gastric function. The conflicting interpretations are largely due to the fact that we did not know until recent years what degrees of variations in gastric secretion are found in apparently healthy, and hence presumably normal, persons, and the further fact that the clinician very seldom knows anything of the gastric secretion in the patient before the onset of the particular ailment that brings him to the doctor's office. But despite this uncertainty as regards the "normal standard," and the usual lack of "controls" on the individual patient, the clinical and experimental literature yields today fairly conclusive answers to the following questions:

1. What are the variations in gastric secretion in normal individuals?
2. Are there primary hypo- and hyperfunctions of the gastric glands?
3. What diseases, not primarily involving the stomach, induce changes in gastric secretion?
4. Do primary changes in the gastric secretion itself produce disease?

A. The variations in gastric secretion in normal individuals. If one studies a large group of persons, otherwise normal, one encounters all the variations in gastric secretion seen in the sick (Martin, Rehfuss and Hawk, Bennett, Bennett and Dodd, Best, Alsberg, Sailor), except possibly the degree of hypersecretion associated with obstruction (functional or anatomic) at the pylorus. Thus one finds in normal individuals hypersecretion and so-called "hyperacidity," and various degrees of hyposecretion or hypoacidity down to complete achylia. One may question the correctness of calling such persons "normal," but they are normal at least to the extent of showing few if any symptoms referable to the change in the gastric secretion. The clinician must henceforth take cognizance of the fact that hypersecretion and clinical hyperacidity as well as hypo- and anacidity are not only compatible

with health, but are found in a considerable percentage of normal individuals. The anomalies in gastric secretion (except gastric retention) exhibited by a patient with any disease may therefore have been present in that individual before the onset of the malady. That complete absence of gastric juice should cause no evident digestive disorder may seem contradictory in view of the important rôle usually ascribed to the gastric juice in the functions of the gut. The explanation is probably to be found in some compensatory mechanisms.

The anacidity may be present without absence of gastric ferments, but usually there is a decrease in pepsin parallel with the hypochlorhydria.

B. The etiology of primary hyposecretion and achylia gastrica simplex. Some cases of achylia have been described as congenital and possibly hereditary (Dauwe), but this seems at present little more than speculation. Others look for the cause in permanent injury to the gastric glands during the life of the individual from such agencies as bacterial toxins, alcohol, too hot or too coarse food, poor teeth, chronic inhibition, etc. (Ramond, Faber, Levinson, Williemse). According to Rosemann there is less storage of chlorides in the gastric mucosa and more chloride salts in the gastric content in persons with hypo- and anacidity. Achylia seems to be more common in women than in men. That the condition is either hereditary or that the injury to the gastric glands is irreparable seems to be indicated by the usual permanency of the condition. This would seem to exclude nervous inhibition as a factor. Leist claims that hypo- and anacidity are associated with a lowered concentration of the blood proteins.

C. Gastric hyposecretion and achylia associated with extra gastric diseases. 1. *Cancer,* irrespective of the location of the tumor, is associated with hypo- and anacidity more or less in direct proportion to development of general cachexia. The hypoacidity usually appears, therefore, late in the cancer history, but there is much individual variation in this regard, indicating that the degree of general cachexia is not the only factor (Palmer). Possibly the initial vigor of the gastric glands may explain this variation. The gastric secretion does not seem to be influenced by the transplanted tumors in rats (Copeman and Hake). Moore advanced the view that the hypoacidity of the gastric secretion in cases of malignant growth is due to an excess alkalinity of the blood. The real explanation is probably more complex.

2. *The anemias,* pernicious, secondary, and experimental, are practically always associated with gastric hypoacidity and anacidity parallel with the degree of cachexia. Hypoacidity is also present in infantile

anorexia and atrophy (Wentworth, Sauer), edema, and very frequently in diseases of the gall bladder (Behm, Rydgard, Rhode, Blackford), in chronic colitis, and in marked hypothyroidism (Boenheim). The gastric hypoacidity may persist after removal of the diseased gall bladder. The hypoacidity is usually associated with increased gastric motility or at least a hastening of gastric evacuation (Voegler). Achylia appears to be induced in some cases by primary disturbances of the heart (Roemheld). Hypo- and anacidity are present in the pregnancy toxemias (Kramer-Petersen). The mechanisms producing the hypoacidity in these conditions are probably varied. Actual asthenia of the gastric glands is no doubt a factor, but bacterial toxins and persistent inhibitory reflexes may also play a rôle. Very little has been done to determine the etiology. It seems fairly certain that the hypoacidity is an effect of the diseases, not a causative factor.

Hypoacidity also appears in pellagra, and in beri-beri in man and animals (Kitamura, LaRue). It probably is one of the effects of all dietary deficiency diseases when the general cachexia is sufficiently advanced.

3. A number of clinical observers (Fleicher and Müller, Einhorn, Strauss, Woelpe) introduced the conception of a "dilution secretion" or "hydrorrhea gastrica" to designate what appears to be an increased volume of gastric juice parallel with hypo- and anacidity, if not actual alkalinity of the gastric juice. This may be seen in rare cases of gastric and duodenal ulcers, but is most frequently observed in cases of acute dilatation of the stomach. Dragstedt has recently shown that the large volume of fluid (usually alkaline) found in the stomach in the latter condition is not secreted by the stomach but comes from the duodenum. The case reported by Einhorn (gastric cancer with cirrhosis of the liver) may be simply a transudate due to partial obstruction and venous stasis. There may also be instances of actual transudate or exudates from the inflamed mucosa in ulcer regions. The case for a real "dilution secretion" or "hydrorrhea gastrica" has not been proved. The excess secretion of the duodenal and the gastric glands in paralytic ileus and acute dilatation of the stomach appears to be due to toxic amines or gastrins absorbed from the gut (Dragstedt).

4. Depression of gastric secretion in fevers. Following Beaumont, clinical observers are in practical agreement that in fevers the deviations from the normal gastric juice are in the direction of hypo- and anacidity. The same has been demonstrated in Pavlov pouch dogs (toxic fevers, thermal fever) (Meyer, Cohn and Carlson). The increased body tem-

perature itself, apart from possible action of bacterial toxins, depresses the gastric glands.

Great increase in the external temperature but without rise in the body temperature is reported to depress gastric secretion and acidity in man, in proportion to the profuseness of the sweating (Cohnheim and Kreglinger, Fischer). There are probably several factors involved in this gastric depression, such as concentration of the blood and inhibition from the mental discomfort of external heat.

5. Hemmeter reported that extirpation of the salivary glands in dogs led to a suppression of the gastric secretion. This has not been confirmed (Loevenhart and Hooker, Swanson). In fact the acidity of the gastric juice may be even slightly increased (but not above the normal limit) after removal of all the salivary glands.

A number of Italian observers have reported a depression (in pepsin) of gastric secretion after splenectomy, indicating some essential relation between the spleen and the gastric glands. These findings have not been substantiated (Inlow). The removal of the spleen decreases slightly the quantity of gastric juice, probably due to a slight interference with the circulation in the stomach.

Clinical observations after resection of the antrum pylori for gastric ulcer (Bilroth operation) indicate that the removal of the pars pylorica depresses the secretion of the fundic glands (see Babkin, Kelling). This needs experimental verification, and seems to be contradicted by the recent work of Ivy and Whitlow on dogs. It is possible, however, that the entire antrum pylori was not separated from the stomach in these experiments. Since extirpation of the pars pylorica hastens the emptying of the stomach, the operation probably shortens the secretion period.

C. Alleged pathological "hyperacidity" and hypersecretion. 1. It is now well established that in gastric and duodenal ulcers, uncomplicated by pyloric obstruction, we may have normal gastric acidity, so-called hyperacidity (that is, hypersecretion) or complete achylia. Since these secretory conditions are found in normal people, it is evident that gastric and duodenal ulcers do not *per se* alter the activity of the gastric glands. But in most cases of pyloric obstruction and consequent gastric retention, the secretory response to foods is prolonged, and there seems to be a tendency to excessive continuous secretion. Actual hyperacidity in the sense of a gastric juice of greater than normal acidity has not been demonstrated in any disease, and probably does not exist. The pathological deviation in acidity is always in the direction toward anacidity.

But actual hypersecretion may exist, although we have no accurate measure of the total gastric secretion in normal persons in the course of a day. It is not less than 1500 cc. and may be double that quantity. When we have partial obstruction at the pylorus the existence of actual hypersecretion cannot be proved, except by some method of continuous drainage of the stomach by the stomach tube. Crohn and Reiss state that ulcer patients may secrete 30 cc. of pure gastric juice in 5 minutes. Even more rapid secretion or 55 cc. in 5 minutes (appetite juice) may be seen in normal persons (Carlson), but this is very rare.

The gastric hypersecretion in ulcer complicated with pyloric obstruction is in all probability due to the increased time of action (gastrin or reflex) of the food in the stomach. The ulcer may also increase the reflex excitability, and actually liberate gastrins and amines into the blood.

Gastric stasis may result from a variety of causes, such as anatomical or functional obstruction at the pylorus, primary gastric asthenia, chronic inhibition, etc. It has not been established that any of these factors can induce gastric hypersecretion, apart from the stasis of food and gastric juice in the stomach. Primary hyperirritability of the gastric gland cells and the local secretory nervous reflex mechanisms; the genesis of gastrin bodies in the autolysis and resorption of tissues, etc., must ever be kept in mind as possible factors. But progress in this field of pathological physiology of man demands greater attention to the continuous secretion and to the limits of its normal variation, as well as to the limits of variation in the acidity of normal gastric juice than has been the usual clinical practice of the past. The notion that gastric content showing 0.2 per cent HCl acidity is the acidity of normal gastric juice, and that 0.4–0.5 per cent HCl is "hyperacidity" should no longer be permitted to confuse the issue. What pure gastric juice (acidity: 0.4 per cent–0.5 per cent) may do in the way of inducing disease symptoms when it acts on sensory and motor mechanisms that are already abnormal is another matter.

In dogs gastric and duodenal ulcers may or may not induce digestive and continuous hypersecretion (Hardt). There is no hyperacidity.

The great amount of attention given in the clinical literature to gastric secretion in gastric and duodenal ulcers is due to the supposed rôle of the juice itself in the etiology and chronicity of the ulcer, the ulcer pains and the pylorospasm.

2. Gastric hypersecretion is frequently seen in appendicitis and visceral adhesions. This has been demonstrated experimentally in dogs

(McWhorter). There is no hyperacidity. Complete pancreatectomy in the dog leads to an excess secretion of gastric juice of normal acidity (Steinberg). Gastric hypersecretion has also been described in patients with toxic goiter (Boenheim), and other nervous abnormalities ("vagotonia") but Hardt found that feeding thyroid extract to Pavlov pouch dogs decreased the gastric secretion. It is probable that the hypersecretion associated with nervous disorders is in part secondary to gastric stasis.

3. The main established facts in the pathology of gastric secretion may be summarized as follows:

1. In otherwise normal persons the gastric secretion may vary from hypersecretion through normal and down to complete anacidity. These variations by themselves do not therefore, produce disease symptoms.

2. In chronic disorders gastric secretion and gastric acidity are decreased on the whole parallel with the degree of general cachexia. The most important factor in this depression is probably the cachexia of the gastric glands.

3. There is no disease known capable of inducing true gastric hyperacidity. The pathological deviations in acid and pepsin concentrations are invariably in the direction of a decrease.

4. Essential hypersecretion (Reichmann's disease) probably does not exist. The factors definitely known to induce hypersecretion are delayed gastric evacuation from obstruction at the pylorus or gastric stasis due to factors that do not at the same time depress the gastric glands. The hypersecretion that is frequently seen in certain so-called nervous disorders has not been sufficiently studied in regard to gastrointestinal motility. If this hypersecretion is primarily of nervous origin, it may be due to depression of the inhibitory secretion tonus quite as much as to excess activity of the appetite nervous mechanism. The marked hypersecretion following a prolonged fast does not seem to induce symptoms of disease.

5. The gastric juice (in normal or greater than normal quantities) can itself produce anatomic or functional disorders only when it acts on tissues or mechanisms that are already pathological.

6. The rôle of the gastric juice in the maintenance of health and in the etiology of disease has been exaggerated, to the neglect of the importance of normal gastric motility.

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CELLULAR IMMUNITY: CONGENITAL AND ACQUIRED TOLERANCE TO NON-PROTEIN SUBSTANCES

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It is now well known that, when acquired immunity is induced in warm-blooded animals to toxins, bacteria, or indeed to any protein substance, this immunity is accompanied by, and at least partly due to, the formation of specific antibodies in the blood or tissue fluids. No such antibodies are formed to non-protein substances, though to some of the latter a high degree of immunity can be acquired. This fundamental distinction has been emphasized by the retention of the word "immunity" as applied to proteins, and by the restricted use of the word "tolerance" as applied to non-protein substances. While this difference in nomenclature is convenient and in part salutary, there is a danger that the distinction may overleap itself and fall on the other side; and this for two reasons. In the first place it has tended often to lead to a tacit and unjustifiable implication that the processes whereby an animal acquires tolerance to non-protein substances do not occur in immunity to proteins; and, secondly, the distinction does not hold good for congenital immunity, because there is no doubt that congenital immunity both to protein and non-protein substances is in many, and possibly in most, cases due to an insusceptibility of the cells themselves to the toxic action of the substance, and independent of any antagonistic effect of the body fluids.

While, therefore, it will be possible in the space of this review to deal only with tolerance to non-protein substances, the object of it is a twofold one. The subject of tolerance to drugs possesses its own interest and importance, but any considerable review of the whole subject has not to my knowledge been attempted since that by Hausmann in the Ergebnisse der Physiologie in 1907. This resumé deals largely with investigations subsequent to that date, and in the necessary selection of material I have chosen rather to consider in some detail only those instances of tolerance which have received some explanatory investigation, than to attempt to mention every known instance of tolerance. The primary object of this review is therefore to give a

general survey, from a scattered literature, of the present state of knowledge of tolerance to drugs. But, in addition, it may serve a purpose of providing a brief statement of the methods, other than by antibody formation or phagocytosis (neither of which has suffered from lack of attention) whereby an organism can acquire immunity, for it is conceivable that even in the case of genuine "immunity" these methods of defence will be found to play a more important part than has hitherto been realized. In any case perhaps this introduction will help to justify the title of the article, for it will be seen that tolerance to drugs is dependent mainly upon properties or activities residing in the cells themselves. All immunity is of course ultimately cellular for antibodies are formed by the cells, so that even the expression "cellular immunity" is one only of convenience.

CONGENITAL TOLERANCE. It is well known that not only different species of animals but different individuals of the same species may show marked differences in resistance to the action of many drugs. This difference in resistance is usually measured by the dose per kilogram of animal that is required to produce a certain physiological or toxic effect—or usually a lethal effect, for death is an important and usually unequivocal end-point. With a great many drugs, e.g., most heavy metals, quinine, chloral, phenol, etc., the M.L.D. per kilogram is very nearly the same for all species of warm-blooded animals; on the other hand with some drugs the difference may be as much as 100 to 1 or more. When one species shows a high resistance as compared with the majority, it is regarded as congenital tolerance in the former; if a low resistance, congenital intolerance or hypersusceptibility. The determination of the causes of these differences is easier in proportion to the degree of difference, and, as the difference between species is usually much greater than the difference between individuals, it is naturally to congenital tolerance in species that investigation has so far chiefly been directed.

Though some progress has been made in explaining—what for medicine generally is perhaps the more important question—why individuals vary in resistance, e.g., as the result of variations in diet, etc., (1), the limited scope of this review will allow consideration almost exclusively of congenital tolerance in species, and even here there are few cases where the cause of this tolerance is yet known.

Congenital tolerance of the toad to toad-poison, and of the toad, snake, and rat to digitalis glucosides. That the toad was "ugly and venomous" had been known for centuries before Vulpian (1854) first subjected the

secretion of the toad's skin to scientific investigation. He discovered its toxic action on the heart and likened it to that of digitalis. He found that the toad is very resistant, though not completely immune, to its own poison and is also much more resistant than the frog to the toxic action of digitalis. Fornara (1871) found that the toad is also resistant to antiarin, and since then it has been found that the toad is resistant also to all other members of the digitalis group which have been investigated in this relation, e.g., strophanthin, apocynamarin and helleborein.

Later it was found that both the rat and the grass snake show, as compared with other animals, a very high degree of tolerance to members of this same group of glucosides. The problems relating to the immunity of the toad to its own venom, and of the toad, snake and rat to digitalis glucosides, are related problems and have often been investigated together. They will here be considered together and in some detail, as this type of congenital tolerance has been investigated with perhaps greater thoroughness than any other.

The immunity of the toad to its own poison and to digitalis glucosides is a specific tolerance, the toad having no comparable resistance to other poisons. Thus Heuser (3) found that the M.L.D. of a large number of drugs, e.g., caffeine, veratrin, chloral, etc., was practically the same both for the frog and toad, though the toad withstood rather larger doses of muscarine and physostigmine. Kobert (4) found that the toad's blood vessels, when perfused, were less affected than the frog's blood vessels, not only by toad poison and digitalis but also by barium. Particular interest attaches to barium because if the toad were resistant also to barium it would almost certainly imply resistance to a particular type of physiological action rather than to a particular type of chemical compound, because, though the actions of digitalis and barium are similar, there is no chemical relation between the two. Heuser (3) however failed to find any increased resistance (as estimated by the M.L.D.) of the toad to barium, and Clark (5) found no increased resistance of the grass snake to barium. In view of Kobert's statement I have repeated these experiments (6) and found that the toad shows no superior tolerance to barium. The M.L.D. is the same for both toad and frog, and the concentrations required to arrest the perfused heart or to produce constriction of the blood vessels is the same in both animals.

The increased tolerance of the rat, as of the toad, applies also to all members of the digitalis group that have been investigated, e.g.,

strophanthin, digitalis, squill. It may reach a high degree; e.g., Hatcher (7) found the M.L.D. of strophanthin for rats to be 100 times that for cats. Clark found the M.L.D. of strophanthin for the grass-snake to be 30 times that for the frog, and that this tolerance in this animal is not associated with any general tolerance to drugs that produce systolic arrest of the heart. From these experiments it is clear that the toad, grass-snake and rat show a singular congenital tolerance to the digitalis group of glucosides which resemble one another in their physical and chemical properties as well as in their physiological actions. The question arises as to what is the explanation of this tolerance.

From a variety of evidence it has become clear that the congenital tolerance of these animals is in every case largely, if not entirely, due to an insusceptibility of the tissues, especially the heart muscle, to the action of digitalis glucosides; and the chief steps by which this conclusion has been arrived at will briefly be summarized. Heuser (3) could find in the toad no evidence of defective absorption, increased destruction in the blood, or increased excretion such as could explain the increased tolerance of this animal as compared with the frog. Hatcher found that the increased tolerance of the rat to strophanthin was not due to difficulty of absorption. Seeing that congenital tolerance in the case of other substances has sometimes been found to be due to increased destruction in the blood and tissues, experiments to determine whether this is true in the case of the tolerance in question have been numerous. Hatcher could recover in the urine nearly all the strophanthin subcutaneously injected in the rat. Roger (8) and Hatcher and Bailey (9) both showed that the mammalian liver did not absorb any strophanthin when the drug was injected into the portal vein. Clark (5) found no destruction of strophanthin by the tissues of the rat or snake.

But the fact that this type of congenital tolerance is due to insusceptibility, in the tolerant animal, of the tissues upon which the drug acts is capable of the following more direct proof. Strophanthin kills by its toxic action on the heart alone. Fraser and Mackenzie (10) showed that the difference in concentration which is toxic for the heart as compared with other tissues of a given animal (frog) is very wide, and there is ample evidence to prove that when strophanthin is injected subcutaneously the heart stops before any other tissue is vitally affected. If the tolerance is due to a tissue insusceptibility, it ought therefore to be revealed clearly by perfusion of the isolated heart. Gunn (11) showed that it required about 30 times the concen-

tration of strophanthin (perfused in Locke's solution) to arrest the rat's heart as sufficed to arrest the rabbit's heart in the same time. As the M.L.D. for the rat was about 30 times that for the rabbit, no further explanation of the tolerance was needed. This was later established for the snake's heart by Clark, who also came to the conclusion that the tolerance of the snake is entirely due to an insusceptibility of the heart to the action of the drug.

The further explanation of why there is this quantitative difference in different animals must depend upon the fundamental method of action of strophanthin on the heart; and this is rather beyond the scope of this review. It may be stated, however, that according to Straub (12) strophanthin acts by altering the physical condition of the surface membrane of the cells, without entering into chemical combination with the cell constituents. Neither he nor Clark (5) could find anything beyond traces of strophanthin absorbed by the perfused heart. The tolerance of these animals to strophanthin must depend, therefore, upon some physical difference in the surface membrane of the cells of the tolerant animals as compared with other animals, and this difference is probably a quantitative one seeing that the same qualitative effects are produced in the heart of both groups of animals but only by different concentrations.

The question of the relation, if any, between the immunity of the toad to its own venom or to digitalis glucosides and the fact that the toad secretes a poison having a similar action has naturally attracted attention. Vulpian (2) considered that the insusceptibility of the toad to its own poison was due to "a sort of accustoming produced by continued molecular absorption of the toxic humour." Phisalix and Bertrand (13) found, by biological assay, toad poison in the blood of the toad and also ascribed the tolerance to self-immunization. This explanation was the more welcome when the modern lore of immunity developed. There are many facts which render this hypothesis suspect, and it may be worth while to examine those facts here as the problem is one of considerable interest for immunity generally.

Kobert (14) stated that the larva of the toad and of the frog are equally susceptible both to toad poison and helleborein and that this was explained by the fact that the venom is developed only in the skin of the adult toad, and that the larva, not having been immunized by absorption of poison, is just as susceptible as the frog larva. I have not found any details of his experiments or of the stages at which the larvae were examined. The statement is certainly not true so far as

the reaction of tadpoles to strophanthin is concerned. I found (6) that the difference between frog and toad tadpoles is just as great as between the mature animals. Frog tadpoles were killed by 1 in 20,000 strophanthin within 24 hours, and by 1 in 100,000 within a week, whereas toad tadpoles continued to live, and indeed completed their metamorphosis, in a solution of 1 in 1000,—an astonishing and unexpected degree of tolerance, even in these circumstances, to a substance the M.L.D. of which for the adult frog was about half a milligram per kilo. If it is true that the poison is developed only in the adult toad, this goes far to disprove the assumption that the immunity of the toad to strophanthin is due to absorption of its own venom.

As I have pointed out elsewhere (11), the immunity of the rat to strophanthin cannot be explained by absorption of any known poison it secretes. Clark (5) found no body resembling digitalis in action in snake's blood and "the explanation of the toad's tolerance does not apply in the case of the snake." Though the grass snake shows an immunity to strophanthin (which acts physiologically like toad poison) the toad shows no increased immunity to snake venom (6). The analogy of the immunity of snakes to snake venom is illuminating. Snakes are highly immune to venom and this has always been regarded as due to self-immunization. But the reasoning is illogical for the grass snake is also highly immune to snake venom, the M.L.D. of cobra venom for the grass snake being over 100 times the M.L.D. for the frog; and yet the grass snake has no venom gland. It might be supposed that, though the grass snake has no venom gland, it might be self-immunized by some constituent in its blood allied to venom. But the cat shows also a high immunity to colubrine venoms (15), and even if it might be supposed to harbor some undiscovered secretion of the nature of a colubrine venom, it is hardly probable that it should also secrete a substance like a viperine venom, and yet the cat is also higher tolerant of viperine venoms (16). Moreover Abel and Macht (17) found that though the toad, Bufo Agua, is relatively immune to the digitalis-like constituent of its venom (Bufagin), it is not similarly immune to the epinephrin in its venom gland; and animals do not seem to become highly immune to their own internal secretions by continued absorption of them. Indeed, if the toad were to become immunized to the digitalis-like constituent of its venom by continual absorption of it, so far from this being a result so axiomatic as hardly to merit proof, it would seem to be an exceptional occurrence.

In short, that self-immunization is a necessary preliminary to congenital tolerance is clearly disproved by the occurrence of tolerance in animals which secrete no poison; that continual absorption of a poison does not necessarily lead to immunity is shown by the fact that animals do not become immune to their own internal secretions; the immunity of the grass snake to venoms shows that the immunity of venomous snakes to venom is probably not, or at least not solely, due to self-immunization. Heterodox though the opinion may be, it seems more in accordance with facts to suppose that, in the case of animals which secrete a poison and which show immunity to it, the congenital tolerance preceded the evolution of the poison secretion, and that possibly it was only in animals which possessed an inherent insusceptibility to a particular type of poison that the evolution of such a poison to a useful degree of development was possible.

Congenital tolerance of the hedgehog to cantharidin. The hedgehog shows a remarkable resistance to a variety of poisons, one of which is cantharidin. Ellinger (18) calculated that one gram of cantharidin is a fatal dose for 20,000 kgm. of man, 500 kgm. of rabbit, and 7 kgm. of hedgehog. He found that this tolerance was not due to defective absorption, for it was true also of intravenous injection. It was not due to chemical change or neutralization in the body because cantharidin could be recovered unaltered in the urine. His experiments showed clearly that the tolerance was due, partly at least, to an insusceptibility of the kidney cells to the action of cantharidin. For example, after an intravenous dose of 0.02 gram in a hedgehog—a dose which produced trifling effects—0.014 gram was isolated from the urine of 24 hours, whereas 0.0001 gram would produce a severe acute hemorrhagic nephritis in a rabbit. As in the case of strophanthus, so congenital tolerance of the hedgehog is due chiefly to an insusceptibility of the tissues in the tolerant animal to the action of the drug.

Many other instances of congenital tolerance are known, some of which will no doubt prove to be of the above type. Heuser (3) found that, to produce motor paralysis, a dose 100 times greater of *curare* is required for salamandra maculata than for rana temporaria. He was unable to confirm the statement of Phisalix that this was due to an antagonistic action of salamander serum. In this case tolerance may be due to tissue insusceptibility. But that congenital tolerance is not always due to this will be seen in the case of the tolerance of the rabbit to atropine.

Congenital tolerance of the rabbit to atropine. Buys (19) incubated emulsions of organs of frog, rabbit and dog with hyoscyamine and then extracted the hyoscyamine from the mixture. He found that the liver of the frog and rabbit completely destroyed hyoscyamine, whereas the liver of the dog did not. As the frog and rabbit were more tolerant of atropine than the dog, the superior destruction by the liver in the former animals pointed to an explanation of this tolerance, and this was the starting point for a series of observations on the power of the liver and other tissues to destroy alkaloids, a large number of these observations being concerned with atropine.

Cloetta (20), regarding the action of atropine as one chiefly on the nervous system, endeavored to determine whether, when atropine was injected, there was a difference in different animals in the amount of retention of it by the brain tissue. He used the rabbit (M.L.D. subcutaneously 0.5 gm. per kilo) as a tolerant animal compared with the cat (M.L.D. subcutaneously 0.03 gm. per kilo). He found no atropine in the brain of either and no difference in the rate with which atropine disappeared from the blood. Seeing that he, confirming Wiechowski (21), was able to recover only less than half of the injected atropine in the excretions, he concluded that atropine must be destroyed in the organism and endeavored to locate this destruction. Incubating atropine with liver and brain emulsions, he found that both, but especially the liver, could destroy atropine and that this power was more marked in the rabbit than in the cat or dog.

Fleischmann (22), following up older observations that a given dose of atropine produced more prolonged action in Berne rabbits than in rabbits from other districts and that this was due to degenerative enlargement of the thyroid common in the former animals, found that the blood (whole or defibrinated) or the serum, of normal rabbits could destroy atropine when left in contact with it *in vitro*, but that the serum of goitrous rabbits did not. Sera of other animals (fowl, calf, man) had not this destructive power. Later (23) he claimed to show that the serum of man destroyed atropine exclusively in cases of thyroid disease. Thyroid extract itself did not destroy atropine. Of the guinea pig, sheep, dog, cat, calf and fowl, only the serum of the first had any marked destructive action on atropine. He came to the general conclusion that the power of the blood to destroy atropine goes parallel with the natural resistance to atropine in different species and in different individuals.

Clark (24) found that in the frog the liver markedly, and the heart and kidney slightly destroyed atropine *in vitro* but not the blood (confirmed by Oettingen); that in the rabbit, the liver and serum destroyed atropine, but no other organ; that in the rat and cat, no organ destroyed atropine, not even the liver or serum.

Metzner (26) confirmed Fleischmann's observation that rabbit's blood could destroy atropine, but was unable to correlate the differences in resistance in individual rabbits with changes in the thyroid. He did agree, however, that the differences in individual resistance which were marked in rabbits did follow differences in individual destructive power of the blood. Danielopolu (27) found that the serum of a rabbit had a less intense action on the pure alkaloid than on the sulphate and that the serum of man, sheep and guinea pig had no action. Doblin and Fleischmann (28) showed that washed red blood corpuscles of rabbits had no power, corresponding to that of serum, to destroy atropine. Schinz (29) found great individual differences in different rabbits in the amount of atropine required to paralyze the vagus and a marked correlation between these differences and the differences in the destructive power of the blood.

Though there are discrepancies in these results, certain points common to them may be taken as established. The liver and plasma of the rabbit and the liver of the frog show a power to destroy atropine, which is not shared by these or other tissues of less tolerant animals. That the liver is more important than the plasma in this respect is suggested by the fact that it is the only organ which possesses this power in the frog—the most tolerant of all animals. Moreover, according to Schinz, to produce the same effect in the rabbit by injection into a mesenteric vein as by injection into an ear vein, a dose twenty times greater is needed. This, if true, would almost certainly imply that, even in the rabbit, the destructive action of the liver is more important, or at least more rapid, than that of the serum. It shows too that the destructive action of the liver, shown by various observers *in vitro*, is operative even in a higher degree *in vivo*. That destruction of atropine occurs *in vivo* in the rabbit, as also that it is an important factor in explaining the congenital tolerance of this animal to atropine, is shown, among other ways, by the experiments of Heffter and Fickewirth (30) who found that though the M.L.D. by subcutaneous injection is greater for the rabbit than for the dog, the M.L.D. by intravenous injection is the same in both. The tissues of both animals are equally susceptible to the toxic action of the drug, but, when the alkaloid is injected sub-

cutaneously, less atropine in an active form reaches those of the rabbit owing to the destruction of the alkaloid which goes on in this animal *pari passu* with absorption, but which does not take place in the dog.

While the general contention of these experiments has been to establish a parallel between congenital tolerance (in individuals and species) to atropine, and the power, peculiar or at least enhanced, which certain tissues in these more tolerant animals possess to destroy the alkaloid, it must be pointed out that, so far as our knowledge goes, the parallel is far from complete. Indeed this would perhaps have more clearly been realized but for a haziness of knowledge as to the relative degree of tolerance to atropine in different animals. An earlier estimate of this was given by Richet (31), and a later one by Clark (24), the latter being a table summarizing the M.L.D. for different animals as found by different observers. This has more recently been reinvestigated by Willberg (32) in a very complete series of experiments, and he has found the M.L.D. of carefully dried atropine sulphate by subcutaneous injection in grams per kilogram to be as follows: white rat, 0.75; guinea pig, 0.45; rabbit (from Luga) 0.5, (from Dorpat) 0.25; white mouse, 0.4; dog (young) 0.23, (full-grown) 0.2; cat, 0.13; fowl, 0.75; duck, 0.25; pigeon, 0.22; bullfinch 0.16. All observers place the frog at over 1.0. In regard to the two most tolerant warm-blooded animals—the rat and the fowl—Clark has shown that no tissue in the rat seems to destroy atropine and other observers have failed to find any destructive power of fowl's serum. As Clark has pointed out, some other reason is needed to explain the tolerance of the rat; and it may also be needed to explain the tolerance of the fowl, though a destructive action of the liver has not been excluded in the latter.

Certainly one of the most remarkable examples of difference of resistance in individuals of the same species is this difference in susceptibility to atropine between rabbits from different districts, and the explanation of this has been shown fairly conclusively to be of the same nature as that of difference in susceptibility between different species. Clark considered that part of this difference might be due to age, as the M.L.D. per kilogram for young rats was about half that for adult rats; but Willberg's experiments in dogs seem to show that in this animal young are more resistant than adults. Perhaps a sufficient number of experiments has not yet been done to determine this age difference which may not be of the same type in all species.

ACQUIRED TOLERANCE: *Acquired tolerance to atropine.* Heckel (33) fed guinea pigs, rabbits and rats for long periods exclusively on the

leaves and roots of belladonna or hyoscyamus without producing any ill effects. He claimed that the animals thereby acquired a slightly increased resistance to subcutaneous injection of atropine (M.L.D. 0.6 instead of 0.5), and also to the local mydriatic action. Similar experiments by Lewin (34) gave similar results.

After the discovery by Cloetta, Fleischmann and others that, especially in tolerant animals, the liver and blood possessed the power to destroy atropine, experiments were made to determine whether this power was increased in animals subjected to prolonged dosage with atropine. Cloetta (35) found that in the case of rabbits which had received increasing doses for 6 or 7 months the destructive function of the liver and blood was to an appreciable degree augmented. This did not occur in the cat. In the case of both rabbits and cats the amount and rapidity of excretion in the urine increased in the accustomed animal. He did not determine whether his "immunized" animals could withstand more than the normal M.L.D. Doblin and Fleischmann (28) treated a dog for six weeks with daily injections of atropine and found no atropine-destroying substance, normally present or developed, in the blood. Schinz (29) reinvestigated the problem in rabbits and got varying results according to the degree of natural tolerance in individual rabbits. Like other observers, he found wide individual differences in atropine-tolerance in different rabbits. He confirmed the observation that these differences corresponded with differences in the power of the blood *in vitro* to destroy atropine. He obtained different results from immunization in the two groups. In rabbits with a naturally high resistance, which co-existed with a high power of the blood to destroy atropine, this power *in vivo* and *in vitro* was markedly increased by immunization, whereas in rabbits with a low natural resistance, whose blood had little power to destroy atropine, this power was not similarly increased. He found also that this atropine-destroying function of the blood could be passively conferred upon less tolerant rabbits and cats, by the injection of the serum from a highly tolerant rabbit. Those results are of considerable theoretical importance and require further confirmation. While it would perhaps be unwise to accept them in the meantime without reservation, so far as they go they point to the conclusion that in animals whose blood originally possesses little or no power to destroy atropine this power cannot be originated, still less increased, by immunization, but in those whose blood can, to begin with, definitely destroy atropine this power can be further augmented by immunization.

That the liver, and especially that the serum, of animals which show either congenital or acquired tolerance to atropine should possess the power to neutralize or destroy the alkaloid raises the even more interesting questions as to what is the nature of the substance responsible and also whether it corresponds in any way to the antibodies formed against proteins (toxins). While no final answer to these questions has yet been given, some progress has been made. An estimate of the capacity of the blood to destroy atropine can be obtained from an experiment of Fleischmann who, using a frog heart arrested by muscarine as a biological test for atropine, found that 1 cc. of rabbit's serum could destroy 0.1 mgm. of atropine in 30 minutes. As to the nature of the active substance, Metzner (26) found that the action of serum was destroyed by heating to 60°C. Clark (24) confirmed this and found that the substance passed through a Berkefeld but not a Chamberland filter, acted slowly and in some respects resembled a ferment. Doblin and Fleischmann showed that it did not pass through a Chamberland filter, was resistant to drying, not dialyzable, not a lipoid, and due to the albumin fraction of serum. They found that it did not behave toward complement like a true antibody. As to the chemical changes involved in the destruction of atropine, Heffter and Fickewirth (30) found that, of the atropine administered by stomach, part is excreted unchanged in the urine, some as tropine and some as an unknown base. The total amount of bases recovered was equivalent to about half the atropine administered. They found that the rabbit can, to a certain extent, combust both tropine and tropic acid, and they therefore suggested that the disappearance of atropine is due to a splitting followed by oxidation of the components. So far therefore as the present state of knowledge goes it would seem that in cases of congenital or acquired tolerance (individual or specific) to atropine, this tolerance is due mainly to the power of the liver, or of the blood, or of both, to destroy atropine, and this power resides in something of the nature of a ferment or ferments—not yet proved to be the same for both liver and blood—which effect the destruction of atropine by splitting it and then oxidizing its component parts.

In regard to the other alkaloids related to atropine, little work has been done in relation to tolerance. Buys found that the rabbit's liver destroys hyoscyamin, and Doblin and Fleischmann that the rabbit's serum can destroy both hyoscine and homatropine. A very remarkable fact and one worthy of further investigation has however been discovered by Van Leeuwin (36), namely, that the monkey exhibits an

extraordinary tolerance to hyoscine, as compared with man. This may very possibly be related to the indubitable individual differences in susceptibility to hyoscine observed clinically in man. Blair (37) has pointed out that in cases of addiction to a combination of heroin and hyoscine a considerable tolerance is required not only to heroin but also to hyoscine. It would seem therefore that acquired tolerance to hyoscine is possible in man.

Acquired tolerance to alcohol. It is a matter of common observation, or even of experience, that the symptoms produced by alcohol become less manifest with repeated use of it. A very recent statement by Mott (38) may be taken as an estimate of the degree of tolerance which can be successfully attained. "As a result of continued use, tolerance can be acquired so that the habitual drunkard may consume, without becoming intoxicated, quantities of alcohol as beverages which would cause well marked signs of drunkenness, or even prove fatal, to a person not accustomed to it."

Pringsheim (39) was the first to make exact experiments to determine the cause of this acquired tolerance. He produced tolerance in rats and rabbits by administration of daily doses of alcohol for a month. He found that, in rats, equal doses of alcohol produced symptoms of drunkenness conspicuously less marked in the accustomed animal than in the normal animal; and, in rabbits, that a dose of alcohol which produced a deep comatose condition lasting for several hours in a normal animal, produced in the accustomed animal only a moderate stage of excitation. It would seem therefore that, in regard to the acquisition of tolerance to alcoholic intoxication, man can boast of no decided superiority over the lower animals. In explanation of this tolerance his experiments led to the following conclusions. Normal and tolerant animals excrete the same amount of alcohol through the kidneys, lung and skin. The feces in both are alcohol-free. The tolerant animal combusts alcohol quicker—in about two-thirds of the time that is required by the normal animal. The percentage alcohol content of the body in acute alcohol poisoning reaches a higher degree in the normal animal than in the tolerant animal—about 66 per cent more. Tolerance to alcohol is therefore to a considerable degree at least due to quicker oxidation of the poison.

The same type of results was obtained by Schweisheimer (40) in man. He compared abstainers, moderate drinkers and confirmed drunkards. He found that, after equal doses of alcohol, the maximum concentration in the blood was lower, the maintenance of the sustained

high concentration—Grehant's plateau—shorter, and the complete elimination from the blood quicker in the tolerant man than the abstainer.

These experiments seem to prove conclusively that tolerance to alcohol is due partly to increased destruction of it,—according to Pringsheim, mainly by the liver. But, as he has pointed out and as would seem probable from other less exact evidence, the same concentration in the blood produces less effect on the nervous system in the tolerant than in the unaccustomed animal. It is probable therefore that an acquired insusceptibility of the nervous system comes also into play.

Hirsch (43) found that in the presence of oxygen and at 37°C. alcohol is destroyed by the livers of animals which have not acquired tolerance. This destruction was apparently due to a ferment for it was inhibited by heating the liver or by the presence of ferment poisons. The liver paste of rabbits that had acquired tolerance was also active, but not more than that of normal animals. Messner (41) however found no recognizable destruction of alcohol by incubating it for 6 hours at 38°C. with tissues in vitro. The last word has not yet been said on the subject for Mellanby (42) states that he has seen no evidence that the rate of destruction is increased in those continually drinking alcohol, nor, if it is taken under constant conditions, of the development of great tolerance.

Pohl (44) was unable to produce tolerance to *methyl alcohol* in dogs, but found that a distinct tolerance, so far at least as the symptoms went, was inducible to *amyl alcohol*. Thus a dog which had been treated with amyl alcohol for over 200 days required at the end of that period 5 cc. to produce the same degree of intoxication as had been produced by 1 cc. in the beginning. It seems probable therefore that the different alcohols vary in regard to the ease with which tolerance can be established to them. Calwell (45) who gave an interesting account of the practice of ether-drinking in Ireland, stated that while the intoxicating dose of *ether* for a novice is from 1 to 4 drams, seasoned topers can consume large amounts up to 2 or 3 ounces. Also while the beginner has to drink water before and after his dram of ether, the habitué perfers his neat, a distinction which he shares with the Highland ghillie. A local and general tolerance can therefore be acquired to ether probably very similar to alcohol tolerance. The same is true of paraldehyde though the habit is rare.

Acquired tolerance to artificial hypnotics. Man will develop a habit and acquire a certain amount of tolerance to nearly all hypnotics. There is perhaps no well-defined tolerance to sulphonal or bromides for, with them, tolerance is complicated by the slowness of their excretion; but with most of the others it is well known that tolerance can be acquired. It is not easy to obtain sufficiently reliable data to estimate the degree to which tolerance can reach, but in regard to the two most important hypnotics, chloral and veronal, it seems unquestionable that men can come to withstand much more than the normal dose, in the case of veronal possibly even lethal doses. Not much work has been done to explain tolerance to these substances.

Wallace (46) gave gradually increasing doses of *chloral* to dogs by stomach, and found that so far as symptoms of depression of the nervous system were concerned, only a slight degree of tolerance was developed, comparable to that displayed to alcohol. Biberfeld (47) investigated tolerance to certain groups of hypnotics. With *amylene hydrate* he could produce no tolerance in rabbits; but in a dog, in which, to begin with, 4 grams of amylene hydrate produced sleep, 8 grams failed to do so after the animal had received repeated injections for 24 days. With *chloral* he found that a dose which produced sleep in a dog ceased to be operative after chloral had been administered regularly for five weeks. He could produce no tolerance to *sulphonal* or to the urea derivatives, urethane, bromural or *veronal.* Bachem (48) gave a dog subcutaneous injections of sodium veronal every three days for about two months but found no evidence of acquired increased destruction as shown by the amount of veronal in the urine. It is not made clear from his account whether his animals really developed any great tolerance.

Chloral is mostly reduced in the tissues to trichlorethyl alcohol which combines with glycuronic acid to form a physiologically-inert urochloralic acid, in which form it is excreted in the urine. Wallace thought that the formation of this combination might possibly keep pace with the increasing amounts of chloral given and by means of this protective agency a tolerance be established. He came to the conclusion however that this combination could not bring about any great tolerance. Biberfeld came to the same conclusion in regard to amylene hydrate, which also unites with glycuronic acid.

Acquired tolerance to cannabis indica. A diminishing susceptibility to the action of Cannabis indica probably occurs with habitual use. Marshall (49) found that a definite tolerance could be produced in dogs.

Fraenkel (50) found that rabbits possess a very high congenital tolerance to indian hemp, in fact they seemed to be almost completely refractory, no effect being produced in them by a dose 100 times greater than was needed to produce typical haschisch sleep in dogs. Tolerance was rapidly induced in dogs to cannabinol, but this tolerance seemed to be limited to the action on the nervous system, for the animals lost much weight during the time of immunization. Beyond the fact of its occurrence, nothing is known of the nature of this tolerance.

Acquired tolerance to opium alkaloids: a. Morphine. It is familiar knowledge that continued use of opium or morphine in man may lead rapidly to a habit and to a very remarkable degree of tolerance. The M. L. D. of morphine for man by mouth has been estimated at about 3 to 6 grains. McIver and Price (51) found the average daily dose of morphine, in a group of people addicted to its use, to be about 15 grains, and in one case as high as 90 grains. Wholey (52) found that one of his patients had been taking daily for six weeks 25 grains of morphine hypodermically, and another 60 grains by mouth. Though some habitués have made exaggerated statements as to the amount of opium or morphine they consumed, it is beyond dispute that, by continued use, man may come to withstand, without fatal results, many times the dose that would be lethal for one not accustomed to the drug. Habit and tolerance are produced by absorption through any channel by which the drug can gain entrance to the body, e.g., by opium smoking or ingestion, by hypodermic injection of morphine, or by snuffing of heroin. Habit and tolerance are induced not only to morphine but at least to those of the opium alkaloids which are closely related to it. Wholey (52) found one of his patients taking daily 17 grains of heroin, though the lethal dose of heroin is less than that of morphine. It seems indubitable that codeine is less likely to form a habit, though Pelz (53) has recorded a case of a man who developed a habit for codeine and who eventually took 25 grains a day. An unfortunate sequel to habit is the occurrence of disagreeable and even dangerous symptoms ("abstinence" symptoms) if the habit is suddenly broken off. No experiments have to my knowledge been made in man to determine the cause of this acquired tolerance, but a very large number has been made on laboratory animals, in certain of whom at all events, especially the dog, a high degree of tolerance can be produced. Only the most important of these investigations will be considered.

Exact experiments instituted to determine the nature of morphine tolerance were possible only when something decisive became known

concerning the fate of morphine in the body. Tauber (54), who gave an excellent account of the previous literature on the subject, first enunciated that in the dog the main channel of excretion of morphine, when injected hypodermically, is by way of the alimentary tract, only negligible quantities being eliminated in the urine. He was able to recover in the feces over 40 per cent of the morphine hypodermically administered. Accepting and confirming Tauber's view that only negligible quantities of morphine were excreted in the urine, Faust (55) proceeded to investigate the changes that occurred in the elimination of morphine in the feces of dogs who had been given gradually increasing doses of the alkaloid and who had developed a well-marked tolerance to its actions. The results he obtained were remarkable. He found that, whereas in acute morphine poisoning about three-fifths of the morphine could be recovered in the feces, the amount found there, when increasing doses were given daily for weeks and tolerance gradually established, rapidly and regularly dwindled until in less than two months no trace of morphine could be discovered in the feces. When the animal was killed at this point no morphine could be found in the liver, spleen, kidneys, or brain. He therefore came to the conclusion that the tolerance which is acquired to morphine is due, not to an accustoming of the tissues themselves, not to a lessened susceptibility on their part to its action, but to the development on the part of the organism of the ability to destroy increasing quantities of morphine.

Cloetta (56) realized that Faust had not revealed by what way or in what organs morphine was destroyed, and also pointed out a serious objection to accepting increased destruction as the sole explanation of morphine tolerance, namely, that this destruction would have to proceed with an almost incredible celerity to explain the complete absence of symptoms in an immunized animal injected subcutaneously with a lethal dose of a rapidly absorbed substance like morphine. He therefore sought a supplementary explanation. He confirmed the observation that the dog excretes large amounts of morphine in the feces, though he found less (23 to 32 per cent) than was found by Faust. In regard to the fate of morphine he found that, injected intravenously, in dogs and rabbits, it disappeared almost completely within five minutes, and completely within twenty minutes, from the blood, a result similar to that previously obtained by several observers. From experiments in vitro he concluded that morphine is not destroyed by blood and must therefore be destroyed by other tissues. When an animal (dog or rabbit) was killed after an injection of morphine and at a time (12 to

60 minutes) when its action was marked, no morphine could be found in the brain and little in the liver. Emulsions of brain or liver to which morphine was added were centrifuged and larger quantities of morphine were found in the brain sediment than the liver sediment. He concluded that brain tissue had a greater affinity for morphine than liver tissue. From the fact that brain incubated with morphine in vitro destroyed more morphine than liver tissue, he argued that the reason no morphine was found in the brain after injection—in spite of the fact that brain had a greater affinity than other tissues for morphine—was due to the fact that brain destroyed the alkaloid. He proved that it was actualy destroyed by the fact that in rabbits and pigeons killed by injection of morphine, less than two-thirds of the amount injected could be recovered in the whole carcass. He found that in a tolerant rat or pigeon (daily injections for 7 or 8 months) morphine disappeared from the body within two days, but not within four hours, after the last injection. The rate of destruction did not appear to be much quicker than in the normal animal though from a comparison of the destructive actions in vitro of tissues of normal and tolerant animals he claimed to find that in the former the brain had acquired an enhanced power to absorb and destroy morphine. Though Cloetta admitted that his experiments did not solve the whole problem of morphine tolerance, they led to an amplification and revision of Faust's view. Briefly, they claimed to show that morphine is actually destroyed, not hidden, in the body; that this destruction is effected largely by the brain itself; but that even in an immunized animal the destruction though greater than in a normal animal is not sufficiently complete altogether to explain the tolerance.

Modification of Faust's theory was further necessitated by experiments of Rubsamen (57). He compared normal and immunized rats in regard to their power to destroy morphine as estimated by the amount recoverable from the whole carcase, and found that, coinciding with the production of tolerance, there occurred an ability to destroy larger quantities of morphine more completely and more rapidly. So far his experiments were in agreement with those of Faust and Cloetta. But this destruction did not occur sufficiently quickly to explain the tolerance, because rats which could withstand 2 M. L. D. with impunity were found to contain in their tissues an amount of morphine sufficient to produce profound toxic effects in unimmunized animals. In regard to the site of this destruction, using an improved technique, he failed to find any destruction in vitro by the brain of the

dog, rabbit or rat, either normal or immunized, being thus in complete contradistinction to Cloetta. He concluded that there were two causes of morphine tolerance, in the first place an increased power of destroying morphine (not explained by in vitro experiments), and secondly, a diminished susceptibility of the tissues to its action—an assumption necessary to explain the fact that, for some time after injection, a quantity of morphine could be found in the immunized animal (showing no symptoms) which would produce profound effects in an unimmunized animal.

Albanese (58) found that the liver of a normal dog had practically no power to destroy morphine, nor had that of an immunized dog (daily injections for 3 months) if the liver were taken a few hours after the last injection; if however the immunized dog were killed 60 hours after the last injection, then the liver possessed an extraordinary power to destroy morphine. Dorlencourt (59) contradicted these results in their entirety, for he found that the liver of both normal and tolerant dogs destroyed morphine (the latter to a greater degree and in proportion to the tolerance developed), but that there was no increase during the abstinence period such as was described by Albanese.

It is difficult to come to a conclusion from results so divergent. Even if these in vitro experiments on morphine destruction were in agreement, they would still have to be reconciled with older experiments of Tauber (54) who found that when blood containing morphine was perfused through the liver and kidney of the pig, practically none was destroyed (other than could be accounted for by unavoidable loss by the method used) though the morphine-containing blood was reperfused through these organs for about a score of times for a period of about two and a half hours.

As if confusion were not already sufficiently confounded, Kauffmann-Asser (60) obtained results which in points of essential importance disagreed with those of his predecessors from Tauber onwards. After a single injection of morphine in rabbits or dogs he found amounts varying from 5 to 30 per cent of the morphine in the urine. In a dog which received daily injections for 17 days, 3 to 13 per cent was recoverable in the urine, and the amount did not fall off in that time. A remarkable result was obtained in the rabbit immunized by daily injections, for the amount of morphine in the urine increased from 5 per cent to begin with to a maximun of 39 per cent on the 12th day, and then fell gradually to nil on the 22nd day. While therefore other observers had proceeded on the fundamental assumption that the

excretion of morphine in the urine is negligible, Kauffmann-Asser found amounts in the urine sometimes even greater than in the feces. These experiments can be disregarded if the recent criticism of Oshika (61) is true, namely, that the method used by Kaufmann-Asser is not applicable to the estimation of morphine in the urine.

These experiments have attempted to solve the problem of morphine tolerance by a quantitative comparison of the amounts destroyed in a normal and tolerant animal respectively, but the problem has been attacked from other angles. Van Egmond (62) found that, in dogs, morphine in doses of 0.04 mgm. per kilo upwards produced a marked slowing of the pulse which he experimentally located to a stimulation of the vagus center. When a dog was immunized by increasing doses until at the end of about three months it was receiving 0.23 mgm. per kilo, it was found that this dose no longer produced the original symptoms of vomiting, narcosis, etc., but still at this time a dose of 1 mgm. per kilo lowered the pulse rate from 120 to 82 for five and a half hours, just as it had done before immunization. In other words, though no tolerance had been gained so far as the vagus center for the heart was concerned, a tolerance had been gained against the symptoms of narcosis, vomiting, etc., to a dose 230 times greater. He drew the only obvious conclusion from this, namely, that tolerance could not be due to increased destruction of morphine, for in that case it would be impossible to explain the fact that in the tolerant animal a minimal dose still produced the same effect on the vagus center for hours after injection as it had originally produced in the unimmunized animal. Increased destruction of morphine at all events of the type adumbrated by Faust and others could not explain a tolerance which was limited to certain tissues only. Essentially the same phenomenon was shown in a slightly different way by van Dongen (63) who found that in a dog that had received, in two months, doses increasing from 10 to 200 mgm. of morphine, while the respiratory center had become tolerant of 1800 times the smallest active dose, the vagus center for the heart was, as van Egmond had found, still as sensitive to the minimal active dose as in the normal dog. He further arranged the descending order in which the centers of the dog acquire tolerance as follows:—pupil, vomiting, diarrhea, narcosis, respiratory center, and lastly the vagus center—which last indeed seemed to acquire no tolerance at all. Especially as this order did not correspond with the order of susceptibility of the different centers in the normal animal, he came to the same conclusion as van Egmond that, whether or not an increased

destruction also takes place, the development of a tissue immunity must also be assumed to explain morphine tolerance. He obtained no comparable results in rabbits, indeed he obtained no evidence of tolerance of any kind in this animal by injections going on for six weeks. Tamura (64) has also found that the vagus center still retains its sensitiveness to morphine in tolerant animals.

Other views on morphine tolerance can be dismissed more briefly. Hirschlaff (65) claimed that the serum of rabbits alleged to be immunized to morphine contained an anti-morphine and that this serum if previously injected into control animals would protect them from lethal doses of morphine. But the numerous experimental errors upon which this conclusion was based were promptly pointed out by Morgenroth (66); and Hirschlaff's results have been contradicted by every subsequent observer, up to, more recently, Pellini and Greenfield (67). Marmé's (68) oxidimorphine theory has also been discredited, though Valenti (69) has revived it to the extent that he claims that the serum of abstinent dogs can produce circulatory disturbances in normal dogs. He has not found what in the serum produces these effects, and his experiments require confirmation.

b. Other alkaloids of the morphine group. Habit and tolerance are common in man to heroin, rare to codeine. Bouma (70) found that in dogs 80 to 90 per cent of codeine injected subcutaneously could be recovered in the urine and feces, the urine containing more than ten times as much as the feces. After prolonged administration no tolerance was produced but rather an increased susceptibility; the organism did not acquire the power to destroy more codeine. Biberfeld (71) found that dogs by repeated administration soon became insusceptible to the sedative action of eucodal or of paracodeine but not to the stimulant actions. Babel (72) found that the brain tissue of rabbits immunized to morphine could destroy heroin but not codeine, and that a pigeon immunized up to 3 M.L.D. of morphine was still normally susceptible to codeine and unable to destroy the latter alkaloid in its tissues. Langer (73) found that in the normal dog heroin was mostly excreted unchanged in the urine, a smaller amount appearing in the feces as an undetermined base, but in a dog immunized by injections for two months no heroin could be found in either urine or feces. He found that tolerance was rapidly produced to the depressant action of heroin but not to the convulsive action. He was unable to produce tolerance to heroin in rabbits.

To sum up the question of acquired tolerance to these morphine alkaloids, it may be said that tolerance is acquired easily to morphine, less easily to heroin, and with great difficulty to codeine. It is most easily produced in man, less easily in dogs, with difficulty, if at all, in rabbits, and not at all—according to Hausmann (74)—in frogs. Tolerance is most easily acquired to the depressant action on the nervous system, far less to other actions. It is chiefly due to an acquired insusceptibility of the nerve cells to this depressant action. Most observers (Faust, Cloetta, Rubsamen, Albanese with morphine and Langer with heroin) agree that there is also acquired during tolerance an increased power of destroying morphine, but as to the details of this process there is no convincing agreement.

Crossed tolerance to hypnotics. Clinical observation seems to have established beyond controversy the fact that people who, by long and faithful use, have acquired an increased tolerance to alcohol, become thereby less susceptible not only to such anesthetics and hypnotics as ether and paraldehyde but also to chloroform, chloral and even veronal. There is also a belief, less well founded, that a person who has acquired tolerance to one cerebral depressant will tolerate larger quantities of other, even chemically unrelated, narcotics. The question of crossed tolerance must obviously throw light on the nature of the original tolerance, and the experiments bearing on it may be discussed here together.

There would be no inherent improbability in supposing that a mechanism which the organism could develop for destroying alcohol more completely, would also effect an increased destruction of ether. Ether however is not destroyed in the body but excreted unchanged. Ether tolerance in alcoholics cannot therefore be due to increased destruction of ether, but rather to an acquired insusceptibility of the nervous system. The presumption is that the same thing occurs with alcohol itself. Apart from the still greater unlikelihood that a destructive mechanism would apply both to alcohol and chloroform, it could hardly operate with sufficient rapidity to interfere with the quick onset of anesthesia produced by chloroform. There is therefore a twofold reason for ascribing chloroform tolerance in alcoholics to an acquired insusceptibility of the nerve cells rather than to an enhanced destruction of chloroform. As it is almost incredible that such a diminished susceptibility should not apply to the substance to which tolerance was originally established, this, apart from other evidence goes far to prove that acquired tolerance to alcohol is due to acquired insusceptibility

of the nerve cells, and that this conveys an insusceptibility to other cerebral depressants.

In regard to other narcotics experiments have been made chiefly by Myers (72) and Biberfeld (75). Myers determined the effects of various hypnotics upon normal dogs as compared with dogs previously rendered tolerant to morphine. He found that in the latter crossed tolerance existed to heroin and codeine. This was manifested chiefly as regards their actions on the respiration and equilibrium, but not on peristalsis. No crossed tolerance was found to cannabis indica or chloral. He concluded that a cross tolerance may exist between closely related drugs but that this is evidenced only on those functions on which the drugs have a common selective action. Biberfeld found, on the other hand, that morphine-tolerant dogs did not acquire tolerance to hyoscine, cocaine, or even to heroin. Though these observations are not sufficiently numerous to enable any comprehensive generalization to be drawn from them, it seems more than probable that acquired tolerance to one narcotic confers some tolerance to other narcotics and that this tolerance is more or less limited to actions which they have in common.

General conclusions as to acquired tolerance to narcotics. The foregoing account of the present state of knowledge in regard to acquired tolerance to hypnotics would have little value beyond a mere catalogue of results, were no more general conclusions deducible from them. A wide survey seems however to reveal certain points of unanimity that may now be summarized, even if they be regarded in the meantime as merely provocative. (1) Among drugs generally, it is chiefly to depressants of the central nervous system that tolerance can be acquired,—to alcohol, chloral, morphine, etc., rather than to, e.g., codeine, strychnine or other alkaloids. (2) When a substance combines a depressant action on certain parts of the nervous system with a stimulant action on other parts of the nervous system or with other physiological actions, it is to the former chiefly or only that tolerance is established. The experiments of Van Egmond, Van Dongen and Tamura with morphine, of Langer with heroin, and of Biberfeld with paracodeine are all in agreement on this point. (3) In regard to these hypnotics therefore acquired tolerance is largely if not entirely due to the fact that certain parts of the central nervous system can acquire an increased resistance to the action of depressants. (4) It may not be accidental to this that tolerance can be acquired the more easily in different animals in proportion to the higher development of the brain, e.g., in the order

man, dog, rabbit, frog. (5) The fact that some degree of tolerance can be acquired to alcohol, chloral, veronal, morphine, cannabis indica, hyoscine, substances with little or no chemical similarity but which agree in being cerebral depressants, suggests strongly that tolerance is more intimately connected with their actions on the nervous system, in which they agree, than with increased destruction of them, in the mechanism of which they must necessarily disagree. (6) Experiments dealing with destruction of alkaloids, etc., by the tissues are not only negatively useless but may be positively misleading unless a very exact method of quantitative estimation is employed.

Acquired tolerance to cocaine. That habit and tolerance to cocaine can be developed in man is only too well known. Tolerance can be acquired very rapidly (76); for example, the daily dose may be increased from 0.1 gram to 3 grams in two months. Grode (81) summarized the literature previous to 1912 dealing with cocaine tolerance. From various published accounts he estimated the M. L. D. for man by hypodermic injection to be from 0.22 to 1 gram; whereas the daily dose taken subcutaneously by an habitué might be as great as 8 grams. There seems no reason to doubt, as some have done, that man can acquire by habituation a high degree of tolerance to cocaine. Little or nothing is yet known of the cause of this tolerance partly because of experimental difficulties arising from the fact that nothing like the same degree of tolerance, if any, has so far been produced in laboratory animals.

Out of a large number of experiments on mice, Ehrlich (77) obtained only a slight and doubtful tolerance in a few animals. Wiechowski (78) could not produce tolerance in dogs (17 injections) nor Von Anrep (79) in rabbits. Ritter (80) after repeated intravenous injections in dogs claimed to observe a diminished intensity of action, but he does no more than mention the fact. Grode (81) could not obtain any evidence of acquired tolerance to cocaine in guinea pigs, fats or dogs, but rather an increasing susceptibility. Mills (82) obtained no cumulative effects in rabbits from repeated injections provided that the intervals between the injections were more than 75 minutes, this being taken to indicate rapid destruction or elimination of cocaine. Rifatwachdani (83) found that the excretion of cocaine in the urine increased by "prolonged" injections but he gave cocaine only for 9 days and his experiments merely show that the quantity of cocaine he gave was not excreted within 24 hours and therefore with daily injections the amount increased daily. He found that when cocaine was left for several hours in a ligatured limb of a living animal, most of it could be recovered,

from which he deduced that cocaine is not to any great extent destroyed by living tissues, but his methods were not sufficiently quantitative to decide this. Wiechowski (78) also found that, of cocaine shaken with liver or muscle juice for 5 hours in vitro, 80 per cent was recoverable. Eggleston and Hatcher (84) on the other hand perfused a cat's liver with defibrinated blood containing cocaine for one hour at 37°C. and calculated, from a biological test, that not less than half had been destroyed.

No conclusions can be drawn as to the nature of acquired tolerance to cocaine from these meager results. If it be true that tolerance to this alkaloid can be obtained only in man, then naturally the solution of the problem can be looked for only from experiments on man himself.

Acquired tolerance to caffeine. As a result of the colossal experiment that has been made, it would be perhaps the general judgment that, in spite of years of indulgence in caffeine-containing beverages, man acquires only a slight degree of tolerance to their effects. No such increase of dose is needed of caffeine as of cocaine, though these alkaloids resemble one another to some extent in their actions on the central nervous system. Experiments on laboratory animals point to a similar conclusion. Bock and Larsen (85) found that, so far as the effects of toxic doses were concerned, only a very limited tolerance to caffeine could be produced in rabbits by repeated injections. This slight tolerance was due not to increased excretion of caffeine but probably to increased destruction of it and to lessened susceptibility of the tissues to its action.

Myers (86) gave rabbits daily hypodermic injections of 100 mgm. of uncombined caffeine for a period of several months and found that, to produce minimal diuresis, twice as much caffeine was needed in these habituated animals as in normal animals. A definite tolerance had therefore been gained to the diuretic action of caffeine. As this was determined by intravenous injection, it pointed to the tolerance being due to an acquired insusceptibility of the kidney cells, though an increased destruction could not positively be excluded.

Acquired tolerance to nicotine. It is beyond question that the majority of men can acquire a certain degree of tolerance to tobacco smoking, and experiments to find out whether a similar tolerance can be induced in laboratory animals to nicotine, and to determine the cause of this tolerance when induced, have frequently been made. As a summary of most of the existing work on nicotine tolerance has already been given (91), and as the results obtained by different in-

vestigators are in most points in agreement, it will be possible to indicate at no great length the present state of knowledge of the subject.

Esser (87), employing large doses, found that in dogs and rabbits a well-defined tolerance to nicotine could be obtained whether the alkaloid were given subcutaneously or by mouth. Hatcher (88) found in rabbits that tolerance was more readily induced by large doses at intervals of 3 days than by more frequent repetition of smaller doses. In any case the tolerance was limited though, once gained, it was retained for some time. Adler and Hensel (89) did not obtain evidence of acquired tolerance in rabbits by intravenous injection.

Edmunds (90), giving nicotine chloride by stomach-tube to cats, found that they became more susceptible (daily doses for a month). He found however that dogs which received subcutaneously injections about every second day, though they showed a similar increased susceptibility for the first six weeks, later began to gain a slight tolerance so that at the end of another three weeks they were able to take twice the original emetic dose without vomiting. With massive doses, after Esser's method, tolerance was apparently quite easily gained. Dixon and Lee (91) found that the liver, and to a less extent the brain and muscle of a tolerant rabbit destroyed more nicotine than the corresponding tissues of a normal rabbit. The substance responsible for the destruction of nicotine was not dependent upon the presence of living intact cells, but in regard to resistance to heat drying, etc., behaved like a ferment. "These experiments show that a certain small degree of tolerance can be obtained to nicotine, and that this is brought by the destruction of the alkaloid. The destruction goes on very slowly, and it can never be accelerated to such a degree that an injection of a poisonous dose of nicotine into the circulation of an animal will lose any large amount of its effect. If the nicotine reaches the circulation slowly and in minute quantities it may be dealt with by the tissues, and this is the condition which we may assume obtains during tobacco smoking."

Edmunds and Smith (92), in replying to certain criticisms which Dixon and Lee had pronounced upon Edmunds' previous experiments, showed that the livers of all dogs possess some power of destroying nicotine but that there were just as great differences in different normal animals as these authors had found between normal and tolerant animals. They therefore did not consider it proved that tolerance was accompanied by any increased power of the liver to destroy nicotine. Clark (93) found that the frog's liver did not destroy nicotine.

So far, then, as nicotine is concerned, experiments are concordant in that they show that tolerance to this alkaloid can be induced in animals by repeated administration—less readily by small than by large doses. The degree of tolerance attainable either in lower animals or in man does not seem to be nearly so high as that induced to morphine. The determination of the cause of the tolerance is therefore more difficult in proportion to the slightness of the tolerance. As to whether the tissues of a tolerant animal destroy more nicotine than the corresponding tissues of an unimmunized animal, the same divergency of results has been obtained as with other alkaloids. That, in the case of nicotine, tolerance is accompanied by increased destruction can hardly be taken yet to be established. No one has made any attempt to discover what becomes of the nicotine when destroyed, e.g., by the liver, though if, by the employment of larger amounts of material, the stages of disintegration could be revealed the results would be interesting.

No work has been done on congenital tolerance to nicotine. The M. L. D. per kilo for the rabbit is stated to be 10 times that for the dog. If congenital tolerance is also due to superior power in the more tolerant animal to destroy nicotine the possibility is promising that the tissues of the rabbit would show a superiority of destructive power over those of the dog—a difference beyond experimental errors in proportion as the difference in tolerance is wide. Hatcher made the interesting observation that young guinea pigs are more susceptible to nicotine than adults, the M. L. D. for the former being 0.15 mgm. per kilo as compared with 0.4 for adults. The explanation of this age difference is not yet forthcoming.

Acquired tolerance to arsenic. It is not necessary here to revive the old dispute about the authenticity of arsenic-eating, for it is now accepted that man can, by habituation, come to tolerate with impunity doses of arsenic which would produce serious effects in people not accustomed to it. Nor need the older literature on the subject be traversed, as summaries of it are already accessible, e.g., by Maclagan (94) and Hausmann (95). The interest which attaches to arsenic tolerance is due largely to the fact that, while acquired tolerance to organic substances is not uncommon, arsenic is almost singular among mineral substances in this respect. Possibly too, the subject has acquired added interest of recent years from the discovery that many protozoa can acquire a high tolerance to arsenic, though there may be little in common between this tolerance and that which is acquired by mammalia to arsenic.

Apart from other difficulties, such as that of accurate quantitative estimation of arsenic, there are two complications which have impeded the study of arsenic tolerance. One is that there are wide individual differences in resistance to arsenic. This has not only been alleged on good grounds for man but has clearly been shown in laboratory animals. The other—a very familiar difficulty—is that the statements in regard to the toxicity of arsenic are so conflicting. Hausmann in his review (95) estimates that the M. L. D. for man is somewhere about 0.1 to 0.2 gram, and this estimate is commonly accepted in textbooks. On the other hand Bradshaw (96), following an older practice, used to give in malaria as much as 120 minims of Fowler's solution (= 0.072 gram arsenious acid) in single doses for three days in succession and "never met with an instance of consequent stomach or any other inconvenience." This was 15 times the maximum usual therapeutic dose and was in fact not far short of a lethal dose according to the usual estimate, and the arsenic in Fowler's solution is in a soluble form.

Cloetta (97) gave increasing doses of arsenic to rabbits and dogs and succeeded in producing definite tolerance, especially to dry arsenious acid. One dog treated for over a year finally received a dose of over 0.4 gram per kilo. He found *a*, that the amount of arsenic in the urine did not rise in proportion to the increase of dose but remained constant, the percentage falling enormously; and *b*, that a dog tolerant of 2.5 grams by mouth was killed by a single hypodermic dose of 40 mgm. He concluded from these facts that tolerance to arsenic was not accompanied by any general tolerance but was due to a diminished permeability of the mucous membrane of the alimentary canal to arsenic. There were two defects in his experiments—first, that only a few irregular estimations of arsenic in the urine were made and it is known that the amount may vary considerably from day to day; and second, that the oft-quoted experiment on the difference in tolerance to stomach and subcutaneous dose was hardly decisive, for the dog received 2.5 grams by mouth one day and 40 mgm. hypodermically the following morning, whereas there ought to have been an interval of a day or more between the two doses. However the contention that there is developed a local tolerance of the mucous membrane of the alimentary canal to dry arsenic has been confirmed in several ways and may be taken to be established.

Hausmann (98), by administering for over a year repeated doses of dry arsenious acid by mouth, immunized a dog to tolerate 0.06 gram per kilo. He found that while the percentage excretion in the

feces diminished from 77 to 30, the percentage excretion in the urine remained stationary at about 5. He concluded that in the course of the development of tolerance the method of excretion was altered and that arsenic was excreted in a combined form not recognizable by ordinary methods.

The question has recently been reinvestigated by Joachimoglu (99). He immunized two dogs (which showed marked differences in resistance) one to 0.056 gram per kilo in 6 months, and the other to 0.025 gram per kilo in 13 months. Arsenious acid was given dry by mouth. He differed *a*, from Cloetta in finding that the absolute amount of arsenic in the urine increased fourfold though the percentage diminished from 15 to 6; and *b*, from Hausmann in finding that not only the absolute but the percentage amount increased in the feces. His experiments therefore showed that with increasing doses the absolute amount of arsenic absorbed and in the circulation increased, and therefore tolerance could not be due entirely to diminished absorption. He substantiated the fact that a local tolerance was developed by microscopic examination of the intestine, the mucous membrane of which in the tolerant dog showed none of the changes which would have occurred after much smaller doses in that of an unimmunized dog. Also a normal dog killed by a large dose of arsenic was found to absorb 31 per cent of the dose given, 8 times as much as he calculated a tolerant dog would have absorbed of the same dose, proving that absorption is diminished in the tolerant animal.

The position now seems to be therefore that tolerance to arsenic can be induced in man or animals when arsenic is given by mouth undissolved, it can be induced much less readily if arsenic is given by mouth in solution, and has not so far been produced by subcutaneous injection. This tolerance is partly due to an acquired insusceptibility of the mucous membrane of the alimentary canal, as the result of which larger doses are required to produce vomiting and diarrhea. About this there seems to be no doubt. Tolerance is also due in part to a diminished absorption of arsenic; the mucous membrane ceases to absorb arsenic in proportion to the increased amount in the lumen, and this effect is probably related to the acquired insusceptibility of the mucous membrane to arsenic. Owing to this protective mechanism there is not a rise in the concentration of arsenic in the blood proportionate to the increase in dose. It seems almost conclusively proved that this diminished absorption goes on pari passu with increasing tolerance, when arsenic is given dry by mouth.

Two points of doubt remain. In the first place it is not certain whether any cellular tolerance is gained by tissues other than the alimentary mucous membrane. According to Joachimoglu, when large doses are given to a tolerant animal the actual concentration in the blood may be high, though not of course in proportion to the dose. As to whether in these circumstances a concentration in the blood is reached which, though innocuous to the tolerant animal, would be injurious to an unimmunized animal is not yet decided. The second difficulty is that no satisfactory reason has yet been given of why repeated increasing doses should in some cases produce tolerance, while in other cases, doses, smaller and given for a shorter time, will produce chronic (? cumulative) poisoning. In the absence of adequate data, speculation on this point would be idle.

Destruction of drugs by the body tissues. One very important problem which has repeatedly arisen in the foregoing discussions relates to the methods by which drugs are destroyed in the body,—whether, for example, congenital tolerance is due to superior destruction of the drug by the more tolerant animal, and whether destruction can be increased by habituation. The experimental investigation of these problems is difficult because it is not an easy task to follow the fate of a drug quantitatively from the time of its injection to the time of its complete disappearance from the body, and this difficulty is reflected in the conflicting results which have been obtained. It is believed that alcohol, atropine, morphine, strychnine (100)—to mention only a few drugs—are partly destroyed in the body, that this destruction is due to ferment action, and that the liver is the organ usually chiefly responsible for the destruction. There is no difficulty in imagining that alcohol may be destroyed in the body, for according to some it is normally present in the blood in small quantities, and in any case is closely related to substances which the body normally destroys. On the other hand it can hardly be supposed that there are *specific* ferments in the body which are lying in wait in the hope that some day a suitable alkaloid will be presented to them for attack. It is highly probable therefore that if alkaloids are destroyed by ferments, the usual business of those ferments is to destroy substances normally occurring in the body, e.g., decomposition products of proteins, amines, etc. It may well be that different animals, according to differences in their diets, etc., may have different powers of destroying alkaloids, and that congenital tolerance may be due sometimes to these differences.

When it comes however to the questions *a*, whether an animal can acquire by habituation a destructive action which it originally did not possess; or *b*, whether it can by habituation increase such a power if preëxistent, the difficulties are greater. In regard to the first question, the destruction of the benzene ring compounds may afford a parallel. The body can destroy the benzene ring of those aromatic aminoacids which enter into the composition of proteins, whereas the benzene ring of foreign aromatic compounds is usually left intact. The condition of alkaptonuria especially shows that when there is a congenital absence of the enzyme which destroys homogentisic acid, this ferment action is never gained throughout life, though the substance to be destroyed is being constantly formed from the food. This seems to be one certain example of the complete inability of the body to form a new enzyme in response to habituation (101).

In regard to the second question, it is quite possible that ferment activity can be artificially increased, because animals fed for a long period on one particular kind of food may develop an abnormal ferment activity for this particular food. The difficulty in regard to, e.g., alkaloids would seem to be this. The ferment that destroys them is one that presumably normally destroys some other substance and it is difficult to imagine how this ferment can be increased by the minute quantities of alkaloid (e.g., of nicotine) which enter the circulation. In other words, one would imagine that there would be even larger fluctuations from day to day in the amount of substance that the ferment normally destroys than would be afforded by the addition of the alkaloid.

At all events these considerations would seem to urge a caution against accepting results which have claimed to show an augmentation of destruction of alkaloids given in small amounts and for short periods and especially those results which claim that a new power of destruction can be originated by habituation.

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DESTRUCTION OF THE RED BLOOD CORPUSCLES IN HEALTH AND DISEASE

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The subject of blood destruction has almost as many ramifications as the blood stream itself; and in entering upon it one must choose a path or perhaps be lost amid data. In the present instance an effort will be made to follow out the physiological processes involved in the fate of the cells, with incidental digressions only for the multifarious agents causing cell injury, and the innumerable consequences thereof.

THE EVIDENCE FOR A NORMAL DESTRUCTION OF BLOOD. So subtly is normal blood destruction conducted and the remains of the cells disposed of that were it not for indirect evidence one might suppose the life of most red corpuscles to endure with that of the body. But this evidence is convincing. The continuous activity of a broadly distributed hematopoietic tissue; the daily excretion through the bile of a pigment nearly if not precisely identical with one of the pigmented derivatives of hemoglobin (220), (221); the appearance of this derivative in old hematomas (224) and in the plasma after hemoglobin injection and liver exclusion (236); the apparently significant association of hemoglobin and bilirubin throughout the animal kingdom (143); the existence here and there in the healthy organism of cells containing erythrocytes in various stages of disintegration; and, not least, the delicate structure of the red cell itself, its lack of a nucleus, the incessant squeezing and buffeting to which it is subjected,—these and other facts clearly prove that blood destruction must be one of the routine tasks of the body. Yet this statement of the case would be one-sided without a mention of the corpuscle's notable aptitude for the existence that it has to lead. Chemical wear and tear are practically negligible in it, as shown by the fact that it uses up almost no oxygen (133), (134), (82); it is almost devoid of autolytic ferments (159), (188), (189), (162); the corpuscular tissue is so constituted as to withstand sudden and considerable changes in the tonicity of the surrounding medium, such as the cell may not infrequently have to encounter; and while yielding is yet so resilient that the disc shape is retained although the cell is forced over and over

again through channels having but a fraction of its diameter. The fluid, too, in which the corpuscles are carried not only acts to protect them against mechanical insult (175), but has other and diverse "buffer" potentialities (235).

THE RATE OF NORMAL BLOOD DESTRUCTION. Were the rate of blood destruction known, one might be better able to judge the adequacy of the physiological mechanisms which have been invoked to accomplish it. But until recently practicable methods have been lacking even for the measurement of the amount of blood possessed by the body at any one time. The development of means to determine plasma volume (99), (129) and total hemoglobin (77), (79) has filled this gap. Yet so aided one ascertains with much exacting labor merely the content of the blood cistern at a special moment. The rates of inflow and outgo, of depletion and renewal, of this content are not touched upon. In the healthy individual these rates must keep pace with each other else the amount of blood would fluctuate more than is the case. It follows that measuring either will give both. All quantitative efforts have centered upon the rate of outgo, that is to say, upon the rate of blood destruction.

The demonstration that hemoglobin injections lead to an increased bilirubin output was a first step in the matter (211). Stadelmann, (206), bringing corroboration, observed that less bilirubin appeared in the bile than was supposedly derivable from the injected pigment and he believed that a part of the latter was utilized in the formation of new red cells. His experiments were only roughly quantitative. That their significance has been overlooked is attested by the many calculations on the normal rate of blood destruction since made which involve the assumption that the bilirubin output furnishes a precise index to the amount of hemoglobin yielded by the destroyed cells. According to one set of figures (76), slightly more than 2 per cent of the body content of corpuscles undergo destruction in every 24 hours; while another (245) has it that five times as much, or about one-tenth the total quantity, is demolished in this time. Recently Brugsch and his collaborators, (37), (38), (39), who almost alone have resorted to experiment in the matter, have followed the bilirubin output in two dogs with bile fistulas, kept suspended in slings, that is to say under highly abnormal conditions, and injected with hemoglobin and hematin. They state that, given a certain formula for hematin, the amount of the substance supposedly yielded by hemoglobin is excreted almost (*fast*) quantitatively as bilirubin; and, with a due recognition of the likelihood of error they go on to figure from the bilirubin output the rate of blood destruc-

tion. Eppinger and Charnass (62) point out that the normal bilirubin yield of fistula dogs is less than half that assumed by Brugsch. On the amended basis they compute that only one thirty-fourth of the blood is daily destroyed. But later authors have in general ignored the correction. And at present the belief,—traceable to the figures of Brugsch and Retzlaff,—is widespread that one-fifteenth of the total blood undergoes destruction each day (1), whatever the animal species.

Experiments lately recorded have been taken to indicate that the quantity of bile pigment manufactured can be greatly increased by carbohydrate administration (237), (239), (240). The protocols leave no doubt that the output of bilirubin was thus rendered more considerable during the period of bile collection. But the said period amounted to only 6 or, at most, 8 hours out of each 24, so the results may have been due to a temporary change in the rate of evacuation of bile pigment from the body rather than to one in the total yield. A like difficulty of interpretation affects observations by the same methods on the bilirubin output following hemoglobin injections (238).

Ashby (6), (7), (8), (9) has procured evidence on the rate of normal blood destruction by following the survival of transfused cells. The blood of individual bulls can be identified with the aid of specifically exhausted isohemolytic sera, and by such means a previous worker had determined the period of survival of cells transfused from one animal (215), (216), to another. The introduced cells were treated from the outset as foreign bodies, and all were removed from the circulation within the extremely short period of 4 to 7 days. But in human beings transfused with blood of a differing but compatible "group," the strange cells would appear to survive for a surprisingly long time. For example, in 10 cases requiring transfusion because of secondary anemia, 40 to 50 per cent of them were still to be found after 28 to 52 days. In a healthy man some persisted for 100 days. There is a periodicity in their elimination. In women they are removed during the menses more especially.

How is one to reconcile such longevity of the cells with the data yielded by the bilirubin output? It has been suggested by some observers (231), who confirm Ashby's transfusion findings, that the alien cells may so far differ from those proper to the host as to be less susceptible to the ordinary processes of destruction. And it is not inconceivable that the stroma constituent of the strange corpuscles whereby they become recognizable does not disappear with the cells but, incorporated in new ones put out by the host, continues for some while longer to

survive. The crying need, though, is not for a reconciliation of figures but for more facts.

Great differences exist in the quantity of blood possessed by the various mammals used in the laboratory, without compensating differences in the unit concentration of hemoglobin (24), (28), (99), (201). And there are other peculiarities, both individual (172) and specific, which find expression in a widely various resistance of the red corpuscles (130), (235), and differing periods of survival *in vitro* (175), (176). That the rate of blood destruction will vary with the species seems certain, though as yet there are no data upon the point. The fact has recently been brought out that the rate in the individual may be profoundly affected by conditions that lie within normal experience (30), (31). Broun finds that exercise of dogs previously kept to a sedentary life brings about a pronounced breaking-down of red cells which becomes evident because the hematopoietic tissue, being taken by surprise, so to speak, is not at once in a position to replace the loss. He terms the state of affairs marrow decompensation. This decompensation fails to occur when the animals are in training, because the blood-forming tissue is then functionally active as shown by the percentage of circulating reticulated cells; while in addition there is actually more of it present. By contrast, when the body for the time being possesses too much blood, as happens in individuals made plethoric by transfusion, the marrow stops work (168). But to review the literature of blood need as a hematopoietic stimulus is unnecessary. The facts to be derived therefrom interlock with those just mentioned in that they show the existence under ordinary circumstances of a delicately balanced coördination between the rates of cell formation and destruction—a coördination which must often act to conceal great variations in the period of cell survival.

THE SIGNS OF AGE IN RED CELLS. Every normal blood specimen is made up of a mixed population of corpuscles of various ages. In keeping with this state of affairs is the wide range of resistance manifested by the cells on test *in vitro*, whatever the test agent be. Were the same cells susceptible or resistant to all such agents one might be justified in concluding that here are the old and here the young among them. But this is not the case.

From Botazzi (23) to Rusznyak (182) there have been investigators who have believed that the resistance of the cells to hypotonic salt solution yields some indication of their age. But Smith and Brown (202), (203) in extensive studies, found nothing to uphold such a view.

Differences in the staining of the individual corpuscles of amphibians with methylene blue have recently been described, and these differences referred to the age of the cells (204). But a like assumption could be made as concerns differences displayed in tests with any other agent.

Ehrlich (60) thought polychromasia a sign of old age in red cells, but it is now generally recognized to be associated with immaturity and with marrow stress. Polychromatophylic cells circulate normally in a considerable number of species (103). Whether they mature in the blood stream remains to be determined. There is a widespread, if vague, belief that they do, and that this happens also to reticulated corpuscles. But the presence of reticulocytes within the large erythrophages of the spleen of the normal guinea pig can be demonstrated with cresyl blue, so it is certain that some at least are destroyed as such (149). Further evidence of this is to be found in the observation that under pathological conditions which involve the production of reticulocytes in quantity, many undergo destruction by fragmentation soon after their entrance into circulation (170).

NORMAL METHODS OF BLOOD DESTRUCTION. Both facts and surmises upon the ordinary fate of the red cell gain a special emphasis from pathological instances, for much of the blood destruction of disease states is consummated by processes that themselves are normal.

Phagocytosis. The presence in the spleen of healthy mammals of large cells containing red corpuscles and the products of their disintegration has been recognized for quite half a century. Shortly after the description by Kupffer of the "Sternzellen" of the liver (225), it was noted that in pernicious anemia these endothelial elements act as hematophages (3). They so act in other diseases entailing increased blood destruction (198), (115), malaria, for example; while similar endothelial hematophages are also to be found in the normal bone marrow, in certain lymph nodes, and scattered elsewhere (157). Under pathological conditions, they may be a prominent constituent of the hemo-lymph nodes (230). In healthy mammals, though, the spleen is their special locus. Often, when blood destruction is increased through disease or by experimental means, an immense number of auxiliary hematophages start into being, as it were. They are elements similar, so far as can be seen, to those that normally engulf red cells, but they either have not previously functioned in this way or so occasionally that their activity has passed unnoted. Sections of the spleen may appear tesselated with such elements, gorged with red cells, and crowded against one another; and the size of the organ may undergo a great increase from this cause (117). In

special instances the phagocytosis is more marked elsewhere. It is especially pronounced in the peripheral lymph nodes during the course of experimental anemia due to *Trypanosoma brucei* (27). At the margin of hemorrhages into the tissues anywhere and from any cause, large endothelial elements may be seen which contain red cells. Exceptionally hematophages may appear in the general circulation.

There is no doubt then that phagocytosis is one method whereby some red cells are disposed of normally, and a great many under special pathological circumstances. To not a few investigators it is the all-sufficing means of blood destruction.

The cells which act as hematophages are, save in special instances, of a single kind, though so widely distributed. They are of endothelial origin. Their relationships and general characteristics have often been discussed (66), (5), (184). Their activity in the ingestion of red cells is merely one expression of a widely exercised function; for they will take up with equal alacrity inert particles of many sorts, bacteria, and some of the so-called vital stains. The most striking evidence for their importance in blood destruction is of recent date.

Most of this evidence has been procured by experiments upon birds. In them hematophages are numerous normally (107) and are situate especially in the liver, not in the spleen as in mammals, a difference which Kupffer himself (226), (227) seems to have been the first to emphasize. McNee (125), (126) has repeated the celebrated experiment of Minkowski and Naunyn (132) showing the failure of hepatectomized geese to develop jaundice when the blood is damaged with arsine. He confirms their result, but not their conclusion that the jaundice is essentially hepatic in origin. True, liver activity is responsible for it, but the cells concerned, instead of being those of the parenchyma, are the Kupffer cells, elements the like of which exist in other organs. They ingest and destroy great numbers of the damaged red cells with a formation of bile pigment. When the liver is taken out the elements elsewhere that are capable of functioning as hematophages assume the task of the Kupffer cells; but they are not sufficiently numerous to form bilirubin in the amount required for an outspoken icterus. McNee concludes that the liver of the goose must be looked upon as a double organ, containing as it does two aggregates of cells, parenchymal and endothelial respectively, that have sharply different functions. Recent studies on the formation of bile pigment in hemorrhagic exudates (220), (221), show beyond doubt,—as, for that matter, did Virchow's demonstration of it in old hematomas (224),—that the substance can be formed outside of the liver.

But the most interesting proof, as suggesting a possibly frequent occurrence, is to be found in the derivation of bilirubin from hemoglobin introduced into a circulation from which the liver has been excluded (236).

Lepehne (113), (114), (116) has tested further the relationship of the Kupffer cells to blood destruction. Assuming that the endothelial cells of the liver, if sufficiently engaged with one sort of phagocyted material might be unable to handle another, he injected collargol into pigeons and geese (116), and, when the endothelial elements had stuffed themselves with it and were presumably incapacitated for the taking up of erythrocytes, he brought about a profuse blood destruction with arsine, after the classical manner. And now, despite the presence of the liver, jaundice failed to develop. The experiment is so engaging that one wishes it were possible to rule out a toxic action of collargol as an explanation of the results.

In rats there are many splenic hematophages. Lepehne (113) finds that after removal of the spleen, the Kupffer cells of the rat liver become active in the destruction of red cells. Pearce and Austin (157) had already noted this vicarious functioning under similar circumstances in dogs, and had found not only the hepatic endothelium engaged in it but that of the lymph nodes as well. And endothelial elements in the hemolymph nodes, as well, may engulf red cells (230). Observations on the occurrence of hemoglobinuria and of hemoglobin-tinted plasma in splenectomized rats (113) require a critical confirmation.

At this writing the precise share taken in blood destruction by the endothelial cells which function as hematophages,—or by the reticuloendothelial system, as the term now goes,—is much in debate among German students of the icterus problem. Aschoff (5) has well presented the case for the importance of the system. He concludes that it is specifically engaged in the working over of hemoglobin, or else in the elaboration of the ferments requisite therefor. Without laboring any point it may be admitted to possess some such rôle in birds, though whether to the exclusion of other "systems" or cells, notably the cells of the hepatic parenchyma, has yet to be settled. But in the case of many, and not improbably all, mammals some other, or at least some additional, means of blood destruction must be sought. For the number of hematophages normally encountered is obviously insufficient for the task they are supposed to accomplish. The cat has so few of them anywhere that prolonged search may be required for the discovery of a single one (178). Yet the cat possesses an active hematopoietic

tissue. Is one to suppose that in this animal blood destruction does not take place *pari passu* with blood formation?

The nature of the stimulus that leads to a normal phagocytosis of red corpuscles can only be speculated upon. Is the process itself in any sense selective? Corpuscles that have been damaged by a variety of agents tend to lag here and there in organs that contain actual and potential hematophages. The rapidity with which they collect in the spleen is sometimes startling, as is the speed of their subsequent ingestion by cells of the organ. It may well be that they are taken up merely as inert bodies that have been rendered in some way foreign to the organism. On this view the normal ageing of corpuscles would involve changes causing certain of them to come to a stop here and there in the body, with phagocytosis as a secondary consequence. The evidence that the red cells collecting in the spleen of some pathological conditions are "physiologically extravasated" (164), (63), (64), (220) is worth remembering in this connection. Whatever the real case there is no doubt that by some hook or crook of function or morphology the spleen often serves as a sort of midden for damaged erythrocytes.

In the blood plasma of normal animals of several species, antibodies have been demonstrated that suffice to bring about, under highly artificial circumstances, a clumping together of the red cells of the same individual (110), (111). It is tempting to suppose that such agglutinins may cause a lagging of old cells in the blood-destroying organs. But the temperature at which the antibodies cause clumping is far below that of the body, and there is no sign of any such happening in the normal organism.

Circulating antibodies, hemopsonins, that induce a phagocytosis specific for red cells (145), occur occasionally in association with various disease conditions (83), notably such as involve anemia (181). Their presence as a rule attracts attention only when elements containing red corpuscles are found in the blood stream. Usually the hematophages are derived from the fixed elements of the reticulo-endothelial system (223), (16); but occasionally all of the leucocytes, even mast-cells, may ingest erythrocytes (181). It has not been possible as yet to study the condition of the viscera in such instances, with a view to learning whether the phenomenon is the peripheral expression of a general activity. Mention has been made of the fact that when the spleen is taken out of an animal in which hematophages are normally prominent, the endothelial elements of other regions take over the task of ingesting red cells. It is perhaps significant that in the first recorded instance of hemato-

phages in the peripheral blood, their occurrence, as also an anemia, followed removal of the spleen (128). In experimental animals subjected to blood destruction or repeatedly injected with dyes or particulate matter, a not inconsiderable number of the endothelial cells of the liver and spleen, engaged for the nonce in phagocytosis, may come loose in the blood (125), (5). Many of them are so bulky with ingested material as to be sieved out of circulation by the capillaries of the lungs.

The plasma of normal man often contains hemopsonins for the cells of individuals of other blood groups (83); and after transfusion of an incompatible blood, a phagocytosis of red cells may occur in the blood stream of the recipient (150). One may suppose that under such circumstance large numbers of the cells are also engulfed in some of the internal organs. For in the nearly analogous cases of animals injected with serum hemolysins or themselves receiving corpuscles from a different species, there is often a great hemophagocytosis, especially in the spleen (117). And in such cases the process comes about, in considerable part at least, through the action of hemopsonins (145).

Fragmentation. Somewhere there must exist in the body, if only for a moment, morphological evidence of the disintegration of every red cell. In several mammalian species possessing few hematophages a search of the organs by perfusion has disclosed a wide distribution of extracellular fragments of erythrocytes (178), still holding their hemoglobin but themselves undergoing further reduction into a fine dust. Similar fragments can be recovered from the circulating blood by differential centrifugation. They are the schistocytes of Ehrlich (58), (59), forms derived from ordinary red cells by subdivision. Can it be that here is a method of destruction worthy of consideration?

The view that corpuscles may normally be threshed to pieces in the circulation is not new (130), nor will it seem strange to anyone who has watched in the living animal, a red cell saddle-bagged at a capillary fork, pulled well-nigh in two, with its bagging portions continually belabored and dragged upon by its passing fellows. Ehrlich thought that the formation of schistocytes comes about as the result of serum changes, and he argued that in the anemias it is purposeful inasmuch as it increases the cell-surface. But the severe occupational strain upon the cells will amply account for the breaking in pieces. When these elements have been damaged in any one of many ways which do not involve immediate destruction, for example, by a serum hemolysin (140), or by the poisonous principle of certain mushrooms (161), or by heat, as during burns (160), (84), (105), an almost immediate consequence is

fragmentation in the blood stream. The changes are tersely and adequately described by Ponfick (160), (161) who was perhaps the first to follow them. Many of the cell fragments produced under such circumstances give up their hemoglobin. As a rule the cell débris rapidly leaves the circulation, lodging in the spleen and elsewhere. Certain of the normal corpuscles of amphibians can be stained while yet circulating, and cells of this sort show a special tendency to fragment (204).

Schistocytes are prominent in the blood picture of many anemias, and are perhaps the more noteworthy because they have given rise to so little speculation. Ashby (7) has published a significant picture of the blood of a recently transfused patient with pernicious anemia, in which the erythrocytes of foreign, that is to say healthy, origin appear sharp-outlined and perfect, in contrast with the irregular, pale or dark, rag, tag and bob-tail corpuscles that are the patient's own possession. Incidentally the picture aids one to understand the strikingly long survival of such transfused cells in patients giving every evidence of continued severe blood destruction. It is, in part at least, a survival of the fit.

May not the schistocytes of the anemias be put forth as such by the bone-marrow? Granted, as concerns some of those found in connection with a marrow so abnormal as that of pernicious anemia. But this does not hold for the cell fragments encountered during regeneration from experimental anemia brought about by bleeding. Under such circumstances large numbers of corpuscles of soft, jelly-like consistency appear in the circulation, many of them soon breaking up into fragments (169). The fragments tend to accumulate in the spleen, but are to be encountered elsewhere as well, though not in the marrow, significantly enough. During the disappearance of compatible blood from healthy animals made plethoric by transfusion, schistocytes become numerous in the circulation and collect in the organs.

If any considerable number of corpuscles are normally threshed to pieces in the blood stream, then an increased functional wear and tear, as when the circulation is quickened, may lead to an increase in the destruction. Poikilocytes and schistocytes are said to appear in the blood of individuals going to high altitudes (60). Mention has been made of the fact that in exercised dogs blood destruction is much more considerable than in sedentary animals (30), (31). An increase in the circulating schistocytes has not been demonstrated. In normal mammals the number of fragmented forms in the blood stream is never great, nor is the accumulation in the spleen impressive.

It has been suggested on histological grounds that the ultimate fate of red cells may be a phagocytosis in the spleen, following fragmentation (2). Lepehne (115) has studied the character of the content of the splenic hematophages in human beings. In individuals dying with evidence of toxic or thermic blood injury great numbers of hemoglobin-containing fragments were present in the hematophages, whatever the situation of these latter, and a lesser number of such fragments was also free in the blood. Lepehne believes that he has discovered a new method of blood destruction, *erythrorhexis* within phagocytes; yet his evidence accords better with the view that fragmentation took place outside of these elements. It is known so to do after toxic and thermic injuries, as already mentioned a page or so back.

The conception that cell destruction in the anemias comes about in considerable part by fragmentation soon after the cells leave the parent tissue involves the view that this tissue in repairing an anemia has to cope not only with the original difficulty responsible for the inferiority of its present yield, but with extra losses consequent on the inferiority. This unfortunate state of affairs probably exists, no matter what the mode by which worn-out cells are disposed of. For it is scarcely to be expected that the corpuscles manufactured under pathological conditions will withstand the exigencies of circulation as long as normal ones. All of which is to say that cell-mortality will continue high until an adequate type of corpuscle is available. The point has received too little recognition in attempts to comprehend the course of blood repair.

The phenomenon of hemolysis in the test tube is so impressive as to have suggested to many observers that normal blood destruction must be effected in some such way. Italian and French hematologists especially have inclined to such a view. They appear to have tacitly accepted the inadequacy of phagocytosis as an explanation of the fate of the corpuscles, and they have been influenced in opinion by the studies of their compatriots on hemolytic icterus, and the favorable results of splenectomy in several blood diseases. The hypothesis according to which blood is normally hemolyzed was first formulated long ago, by Botazzi (23). According to Banti (12), (13), whose conceptions have been widely adopted, there exist in the body "hemolytic organs," of which the spleen is the most important, which may be excited in various ways to great activity and are so excited in the hemolytic icterus above referred to. Many experiments are on record in which normal hemolysins feature, and some demonstrations as well of free hemoglobin in blood from the splenic

vein; but the difficulties of interpretation are great. Injections of splenic extract are said to lower the number of circulating red cells. The whole subject has been reviewed by Pearce and Krumbhaar (158),—who have made many observations in connection with it,—and recently by Eddy (57). At the present time the thesis that hemolysis is concerned in normal blood destruction must be looked upon as not proven.

To sum up, only two normal methods have thus far been discovered whereby worn-out red corpuscles leave the circulation, namely, phagocytosis and fragmentation. The amount of phagocytosis normally going on in the body is often insufficient to account for the disappearance of many cells whereas a constant slight fragmentation throughout the circulation will readily do so. Both processes may quite possibly be of little consequence as compared with some other, unrecognized as yet. One is privileged to believe as one wishes about the matter, but scarcely to draw conclusions.

Phagocytosis and fragmentation of the corpuscle have been discussed at length because they are processes natural not only to the healthy, but to the diseased organism in which large quantities of blood are rapidly broken down. They serve often in this latter connection as the means of first resort. But there are a host of pathological conditions in which destruction of the red cells comes about by methods essentially morbid and frequently of extraneous derivation. These methods will be but briefly dwelt upon.

Abnormal methods of blood destruction. Many forms of injury to the red cell that are encountered in man, under present industrial conditions, are so bizarre as almost to rank with the wantonly experimental. They are not ensconced in the life of the species like injury by the malarial parasite and by bacterial organisms. And they need be mentioned here only as they throw light on physiological laws. This they sometimes do. But it is the weakness of much of the current experimental work on blood diseases that it is carried out with injurious agents the effects of which on the red cell and on the animal body can only be regarded as highly artificial.

Hemolysis. The chemical and physical agents which bring about a liberation of hemoglobin from the corpuscles are legion; and various are the ways in which they compass the result (235). Several attempts have been made to follow, through the darkfield microscope, the morphological changes incident to hemolysis. They have been so far successful as to have shown that the changes are not always the same (53), (186). The resistance of the cells to any one hemolysin, as in-

dicated by the freeing of pigment from the corpuscle, is often no index to that of others (183), (104). What agent then shall one take as an indicator of the age or condition of the cell? Manifestly the facts give no warrant for taking any. Yet it is current practice to choose an hemolysin for clinical tests of cell resistance, usually one that is readily available and easily manipulated. And our ignorance justifies the practice. Cell peculiarities have been demonstrated thereby in not a few conditions, and although their meaning awaits elucidation, the discovery of them has aroused speculation when it has not aided diagnosis.

That hemolysis may on occasion take place in the animal body has been realized ever since the disastrous seventeenth century efforts to invigorate the old and sometimes wicked with blood from the young and sufficiently innocent. The literature of the time contains many succinct descriptions of the events which followed the giving of a wrong strange blood (109). The studies of Bordet and Ehrlich on serum hemolysins rationalized these happenings and set a throng of investigators upon the idea that the activity of such antibodies would explain blood destruction in general (185). Unfortunately, the existence in the normal human organism of isolysins and isoagglutinins was not recognized for several years (112), (137); and many findings which seemed significant have now been traced to them. At the present day the destruction of one disease at least, paroxysmal hemoglobinuria (54), is known to be produced by a serum hemolysin having the character of an antibody; and in several other maladies in which there are signs of a great breaking down of corpuscles, notably in pernicious anemia (174), (242), (228) and some forms of hemolytic icterus (46), (212), auto-hemolysins have been encountered. The effect of isohemolysins is only too frequently witnessed in transfused cases. No further comment would be called for in this connection save for a curious development of a sort essentially modern. The technique of transfusion methods has been pushed to perfection without a proportionate understanding of the results of the procedure; and in consequence there are now on record not a few instances in which, through frequent transfusions, immunization of the human creature against bloods previously compatible has been unwittingly accomplished (22), (208). Often the immunized individual can now no longer be helped by the transfer of corpuscles from anyone, but is greatly harmed instead. The newly developed lysins may or may not be demonstrable *in vitro*.

Under pathological circumstances substances potentially hemolytic but

normal to special organs or tracts may escape from their usual environment into the general circulation and damage the red cells. The bile of most species is hemolytic, by reason of the cholates that it contains (119); and the anemia so often associated with prolonged jaundice from obstruction has been attributed to lysis by bile salts, though, as we now know, the plasma acts to prevent this (18), (119), (199). The anemia of nephritis, as of certain other diseases, is not inconceivably due in part to lysis by retained metabolic products. Hemolysins have been demonstrated in necrotic tissues (232); and blood destruction in some patients with large necrosing tumors may conceivably be caused by such bodies. Chronic poisoning with toluylenediamine results in the formation of hemolytic substances by autolysis (98), (127). The suggestion that fatty acids may be responsible for a lysis of cells in patients has led to much work (101); but it is not supported by the most recent findings (52). The need for a rational therapy of pernicious anemia, coupled with the evidence for hemolysis in the disease, has led to a wide extension of knowledge as regards lytic agents which, one by one, through successive waves of investigation, have been proven guiltless in the matter (153).

Banti's view (12), (13) that blood destruction is caused by a hyperactivity for hemolysis of certain organs, notably the spleen (71), (73), (74), (75) has been discussed in its relation to normal blood destruction. Whether the hemolysins which can not infrequently be separated out of the tissues (69), (21), (147), (148), (233), (234) play any rôle in body processes is dubious, to say the least. Hunter (94) is largely responsible for the stimulative idea that blood destruction in pernicious anemia comes about through the action of a lytic substance absorbed from the intestinal tract. The finding of Bunting (43) and others (120) that blood pictures much like those characteristic of the disease may follow upon the repeated intravascular injection of hemolysins; Tallqvist's (210) observations on the anemia sometimes developing in individuals who harbor the tapeworm *Bothriocephalus latus;* and the demonstration of the permeability of the intestinal wall, not only to some of the simpler hemolytic poisons (96) but to serum antibodies (67), are alike encouraging to such a view. Yet there are investigators who maintain that pernicious anemia is not a disease of blood destruction at all. They pit isolated facts or ingenious hypotheses against the mass of data which attests to a havoc among the red cells.

The discovery that certain microörganisms will produce lysins (hemotoxins) (213), (214), (124), (163), (164) under appropriate conditions

has a potential bearing not only on the anemias of obscure origin but on the serious blood changes that occur in many infectious diseases. In most such instances we do not yet know whether an increased destruction or a lessened formation of cells, or a combination of both, is to be blamed. In the average case of scarlet fever, for example, a drop in the red count of about one million cells is said to occur (90), and with that said, knowledge upon the matter has been nearly summed up (108). Yet it should be possible, by methods now available, to determine where in the blood cycle the trouble occurs.

Some of the hemotoxins, those truly deserving the name, behave like genuine toxins, inducing in the animal a formation of antibodies which may on occasion have a high titre (213), (214). The recent work of Bull on the toxin of *B. welchii* (40), (41), (42) furnishes a case in point. The nature of many other hemotoxins is only vaguely apprehended. There is good reason to suppose that the malarial organism (15), (29), (244) and numerous other animal parasites give off substances that cause lysis (209), (210). But the literature on hemolysins of extrinsic origin,—a literature of stupefying dimensions,—cannot be reviewed here. Work with these agents has not of late greatly furthered an understanding of the laws of blood destruction within the organism. In 1877 Ponfick (160), describing of the effects of mushroom poisoning in dogs, set forth in a couple of paragraphs nearly all that we now know of the ways in which red cells go to pieces when acted upon within the body by an hemolysin. He observed lysis and fragmentation occurring together, and a local deposition of the cell débris with subsequent phagocytosis of it and of cells still intact.

The failure to demonstrate serum or other hemolysins in clinical conditions in which there exists good reason to suppose them active is evidence rather for the inadequacy of current methods of test than for conclusions on the state of affairs within the body. For example, an amount of serum hemolysin, which suffices to lake but a few cubic centimeters of blood *in vitro* may, when introduced into the organism, give rise to great destruction (139). This comes about in part through a transfer of the hemolysin from cell to cell, and in other part (135), (136), (138), (139), through an increased fragmentation and phagocytosis of corpuscles to which they become liable without any liberation of pigment having taken place. Furthermore, there can be no doubt that an injury insufficient to produce hemolysis under test-tube conditions may bring it about in the animal body. The development of methods which

will enable one to recognize *in vitro* when red cells become non-viable rests as an urgent task upon hematologists. At present workers employ, of necessity, the crudest possible criterion, namely, lysis.

There are yet other sources of difficulty in the demonstration of hemolysins in disease states. Some of them never enter the general circulation. The one which is responsible, presumably, for the anemia associated with the broad tapeworm (*Bothriocephalus*) disappears from the blood of animals within 15 minutes after injection, as experiment has shown (209); and yet it does not fail to exert for a much longer period its destructive influence. Some agents give rise to hemolysis only as the result of changes they induce in the tissues (98). There are modifying serum factors and others of dilution and proportion which are not taken into account in test-mixtures as ordinarily set up,—factors which vary greatly from individual to individual. But it is unnecessary to enlarge on our inability to copy body conditions.

Hemagglutination. Landsteiner (110), who first demonstrated the presence of autohemagglutinins in the plasma of normal animals, showed that these bodies have the same essential characters as the iso- and heteroagglutinins, but are much weaker, and, as already mentioned, act to cause a clumping of cells only under highly artificial conditions. Agglutinins have also been found in extracts of the normal corpuscles of some species (102), (171). The question arises whether under pathological conditions such auto-antibodies ever bring about blood destruction. There are facts to suggest that they do:—In diseased human beings they are occasionally of high titre (89), (48), (241) and are found in association with anemia. Yet it is not always certain that the anemia may not be a cause for their occurrence rather than an effect of it. For autoagglutinins appear in animals rendered anemic by bleeding (170), as also when blood destruction is increased by the repeated injection of compatible corpuscles (180), (171). It is claimed that when blood kept outside of the body for several days is returned to the individual whence it came, autoagglutinins develop (121). The fact may be granted but its interpretation remains uncertain. For the temporary removal of the blood entails a temporary anemia such as may itself lead to an appearance of agglutinins.

Isoagglutinins are responsible for some of the untoward effects of the transfusion of incompatible blood (150), (151). Certain disease-producing bacteria give rise to agglutinins effective against the corpuscle (244). Serious changes in the organs as a result of hemagglutination

have been described (70), (154), (155). The nature of these changes is such that any considerable participation of agglutination in pathological blood destruction would surely have been discovered ere now.

CELL DESTRUCTION THROUGH PIGMENT CHANGES. On pathological occasion the hemoglobin of erythrocytes may be so combined with or altered that the cells become more or less useless as oxygen carriers. The combination may be reversible, and, in one such case at least, that of carbon monoxide poisoning, the affected corpuscles continue to circulate until restored to their normal condition (79), (146). When a permanent change has been effected, as to methemoglobin, they may rapidly leave the blood stream although still intact (207). A profound anemia may thus develop within a few minutes. The fate of the changed pigment has not been traced. Cole (49) and others (45) have brought out the fact that infection with the pneumococcus can lead to some formation of methemoglobin. Usually this is not found free in the serum, though Van den Bergh and Engelkes (222) have described both methemoglobinemia and sulphmethemoglobinemia accompanied by hemolysis. In many forms of fulminant blood destruction hematinemia has been noted (193), (194), (195) as the result of changes in the hemoglobin after it has left the corpuscle.

THE INFLUENCE OF CONGENITAL PECULIARITIES ON BLOOD DESTRUCTION. In an occasional human being some of the erythrocytes habitually put forth by the marrow are disc shaped (55), (56) or even of an attenuated sickle shape (61), (85); and there may be a family tendency to the condition and an anemia associated with it. Other individuals are born with a tendency to jaundice of hemolytic character (212); and here again a family influence is often visible. The peculiarity may assert itself merely by the presence of slightly more bilirubin in the blood serum than is normal (*cholèmie familiale simple*); or again it may lead to pronounced jaundice and anemia persisting throughout a long life. Autoagglutinins and hemolysins have been demonstrated in individual instances (46). A noteworthy, almost unique, character of the erythrocytes is their lessened resistance to lysis by hypotonic solutions (44). Resistance of the sort is remarkably uniform in most diseases and in normal states as well, though, strangely enough, it is enhanced in the obstructive forms of jaundice.

The conclusion cannot be avoided that some congenital factor is active in all the conditions mentioned. But the nature of the factor, or factors, affecting the corpuscles has thus far eluded determination. There is evidence that plasma abnormalities may be the cause for sickle-

shaped red cells, and some observers would lay the various forms of family cholemia to a liver derangement (17),—though a marrow abnormality affecting the character of the cell output seems more likely. Whatever the trouble its ultimate results are in one respect at least often the same. There is increased blood destruction with splenic enlargement. The steps in the process of destruction have not been followed. Banti's explanation—that there is a splenic hyperactivity leading to hemolysis—has already been discussed.

Cell destruction by extravasation. Erythrocytes confined between ligatures upon a vessel break down much more slowly than do others escaping into the tissues (192). Tissue ferments have been held responsible for the difference. It is certain that the great majority of the red cells of hematomas do not regain the circulation but disintegrate on the spot. Kretz (106) suggested many years ago that in some obscure cases in which the liver contains an abnormally large amount of blood pigment, repeated small hemorrhages will account for the finding. The anemia of severe cases of purpura is clearly traceable to the repeated local extravasation and disintegration of blood. Whether there are other anemias consequent on long-continued occult hemorrhages within the body has not been ascertained.

The course of events in hematomas is one of the oldest themes in modern pathology (224). Recently the alterations in the blood pigment have been studied, with improved methods, by Van den Bergh and his associates (219), (220). They emphasize the similarity to changes taking place in the spleen in some of the blood diseases. Pribram (164) had noted that in chronic passive congestion of the spleen there is a marked local breaking down of blood; while Eppinger (64) had laid stress on the vascular peculiarities of the organ in pernicious anemia, advocating the view that the destruction of red cells results from what is in effect a constantly recurring extravasation into the splenic pulp. And now Van den Bergh has brought chemical evidence for a "physiological extravasation" of red cells, not alone in pernicious anemia but in other conditions.

Destruction as affected by plasma changes. According to Hamberger (81)—and the idea has become a familiar one to students of the chemistry of the blood—however simple a plasma change may be, its consequence is a change in the chemical constitution of the red blood cell. There can be no question but that many plasma changes which take place within the body must make for or against the survival of the corpuscle, if not by causing interior alterations, then by acting from

without. The fluid of the normal blood acts to protect the cells against mechanical insult, and from the effects of many hemolysins (235). But may not the highly abnormal, only slightly viscous plasma of pernicious anemia (144) fail in some degree to ward off mechanical harm to the cells during their incessant round of the body? A decreased power of the plasma in this disease to protect red cells from hemolysis by soaps has lately been described (47); but that the power itself is of practical moment has now to be proven. An attempt has been made to group with the various forms of congenital jaundice those blood states characterized by the presence of sickle or disc-shaped cells as well as the others in which autoagglutinins or hemopsonins have been found, and to attribute all to plasma changes (229).

THE RATE OF PATHOLOGICAL BLOOD DESTRUCTION. The possibility that the destruction of red corpuscles may sometimes slacken in disease has been scarcely considered, so generally is it hastened. Yet there are data to show that in rabbits transfused and having need of the blood thus provided this lasts longer than in normal animals (26). The finding may possibly help to explain the protracted survival of transfused cells in some human beings (7), (8), (9). The assertion has recently been made that the degradation of hemoglobin by the liver can be delayed by poisons acting upon the organ, and that a polycythemia then ensues which is the result of decreased blood destruction (88). Almost the whole of previous knowledge speaks against such a happening.

The increased blood destruction of many pathological states is accomplished, as it will do no harm to state again, by methods that are normal in themselves. Hunter assumed as much in his studies of the fate of the corpuscles in dogs made repeatedly plethoric by transfusion (93). He observed that an ability to break down the blood with speed was acquired by the animals, a finding confirmed by later workers (25), (87). The destruction, in so far as it is brought about by antibodies directed against the alien cells, must be considered pathological. But the demonstration that exercise hastens corpuscular disintegration in normal animals (30), (31) suggests the thought that, in many diseases unrelated directly to the blood, the physiological apparatus concerned in the destruction of its cells may be affected.

Current estimates upon the pace of cell destruction in disease are based upon the same insecure premises and data as are those for health. The amount of urobilin in the stools has been assumed by many workers to yield an index to it (62), (243), (167); and certainly there are

clinical observations attesting to a usefulness of the substance in this connection. But urobilin is derived from bilirubin, which, as we have seen, may not represent destroyed blood quantitatively; while furthermore the amount of urobilin which can be got from normal stools by present methods is far below that which should be yielded by the bilirubin turned into the intestines (62). Eppinger and Charnass, admitting that this prevents inferences on normal blood destruction, hold nevertheless that calculations from the quantity of urobilin found under pathological conditions can at worst lead only to an under-estimation of the number of cells destroyed. This quantity is so great in some cases of congenital hemolytic jaundice that a turnover of the entire amount of blood once in every two or three days would be required to account for it (62). A recent reviewer, discussing the possibility of a similarly swift destruction and replacement in pernicious anemia, finds it so far out of the question as to reduce to absurdity the general reliance upon urobilin figures (239). Yet when one considers how rapidly blood broken down within the body may be replaced, how little the total quantity sometimes is in the diseases just mentioned, and how widespread and active the hematopoietic tissue, this seems not necessarily to follow.

The appearance of punctated cells in individuals given small doses of lead by mouth has been utilized in a study of the period of survival of corpuscles in human beings with malaria (50). Some of the punctated cells survive for 2 days at least. The method is highly limited in application, needless to remark.

A destruction of the greater part, if not of the whole of the blood can be brought about before life is extinct by the use of physical and chemical agents. But these agents and the physiological situations they induce have little interest in the present connection.

Disposal of the cell material. The capabilities of the organism to deal with damaged corpuscles are very great. Cells that have been injured by experimental means usually leave the circulation practically at once. It might, indeed, almost be set down as a general rule that their continued presence in the blood stream means that serious destruction is still going on, and that the animal will die if it be not soon checked. This rule would fail to hold in connection with the damaged corpuscles of disease states. The distorted or fragmented cells of many anemias, unlike those occurring in consequence of the action of poisons of extrinsic origin, must certainly go the round of the vessels for some time, since if they disappeared promptly there would often be little blood left. And

corpuscles carrying the malarial plasmodium may function until their substance had been almost wholly replaced by the parasite.

The morphological changes undergone by phagocyted red cells have often been followed. The view is widely accepted that normally the hemoglobin derived from them is passed on from the spleen to the liver. This involves the assumption of some sort of intracellular hemolysis. But the histological evidence is wholly against such an occurrence. The red corpuscles within hematophages are never visibly decolorized, nor do they merge into larger hemoglobin-tinted globules such as form after the ingestion of cells already hemolyzing (177). Instead, cell fragments of various size are regularly seen, all of them smaller than the ordinary erythrocyte. They are at first rounded and give the staining reactions for hemoglobin, but are sometimes distinguishable by other means from fragments of extracellular origin (178). They undergo a gradual conversion into angular brown granules of hemosiderin, and one gains the impression that the changes take place at a slow rate. The tardy utilization of the iron deposited in splenic phagocytes, as compared with that in the hepatic parenchyma, is in keeping with this impression (142). Biliverdin has been recognized in the hematophages of birds (5).

The normal fragmentation of red cells progresses until they are reduced to a fine, hemoglobin-containing dust. Some of the fragments and dust lodge here and there, especially in the spleen (178). But where and how the ultimate separation of hemoglobin and cell fabric takes place remains to be discovered. Not improbably it occurs throughout the circulation, and the accumulation of schistocytes in the spleen is incidental to the peculiar vascular arrangement of this organ. The fact that bilirubin can be formed from blood pigment almost anywhere in the body fits in with such a conception.

Some part of the cell-fabric, or stroma, has an immunological peculiarity (185) and might conceivably be traced. It has been separated from the cells in the test tube (11). But the conservation of the stroma substances would seem to be of little importance to the organism. McMaster and Haessler (122) were unable to deplete the body of them to such extent as to interfere with the formation of new erythrocytes. It is a common observation that the production of stroma regularly outruns that of hemoglobin during recovery from the anemia of hemorrhage. The statement has been made that such repair may be hastened, both as regards cell number and hemoglobin percentage, by the injection of lipoids derived from the corpuscles of an animal of another species (100).

Gross lysis of cells in the blood stream brings with it a large train of pathological consequences. With the more remote we are not concerned. Observers have likened the immediate shock to that of anaphylaxis (20), (22) and some believe that it is referable to similar causes. Blood crises characterized by the appearance of immature cell forms in circulation, and closely resembling those of pernicious anemia, occasionally follow upon the transfusion of an incompatible blood (152). The factor responsible for them has yet to be identified. Free hemoglobin seems not to be toxic (14), (200), save perhaps in occasional disease instances. Artificially prepared globin, though, may cause serious disturbance, at least in experimental animals (190). And the stroma from the cells gives rise, in addition to fever, to serious disturbances in some species (14),—not in others (20),—by lodging here and there and mechanically obstructing the blood stream. It is the better enabled so to do because it induces clotting (118). It collects for the greater part in the spleen and lungs. When blood destruction has been brought about with toluylenediamine in splenectomized animals the "shadows" of hemolyzed cells circulate for a much longer time than is the case in controls not deprived of the spleen (97).

Free hemoglobin, hematin and bilirubin may all be met with in old hematomas; and they may be associated together in the large spleens of patients with some of the blood diseases (220). The formation of bilirubin in the perfused normal spleen has recently been described (65). That the pigment from damaged cells accumulating in the organ may undergo the same changes as in hematomas one can readily believe. Van den Bergh's view that the destruction of corpuscles in pernicious anemia comes about by a "physiological extravasation" into the splenic tissue is based on this assumption.

Free hemoglobin has not been demonstrated in normal plasma, although in some species of animals (217), notably in man, bilirubin is present in a concentration which permits quantitative observations (91), (218), (220). Injected hemoglobin is rapidly removed from the blood stream, notably by the liver. Crystals of it have been recognized in the hepatic parenchyma of healthy animals (32), (86). That it cannot be immediately utilized again, as such, for new-formed corpuscles is indicated by the tardy changes in the color index of the blood of animals injected with it when they are putting out abnormally pale cells to repair losses from hemorrhage (122). Indications exist of intermediate stations (*Zwischenstationen*) (191) where it is worked over. The fact that it is manufactured with more difficulty than the rest of the cell has already

been referred to. In view of these facts there is nothing surprising in the circumstance that the body possesses an excellent arrangement for the retention of the more important part of such pigment as may accidentally be liberated from corpuscles (141). Considerable quantities must accumulate in the plasma before any escapes into the urine (51); and the liver acts to prevent the necessary accumulation by swiftly removing it from circulation (10), (156). In rabbits given a specific hemolysin, more than one-half of all the blood may be destroyed within 48 hours and yet no hemoglobinuria occur (140). But in human beings long subject to blood destruction the pigment may appear in the urine after injections of it which in healthy individuals would have no such result (200). Either the liver or the kidney may conceivably be at fault in such instances.

In animals rendered anemic by blood destruction within the body the loss is repaired more rapidly and satisfactorily than when depletion has been accomplished by bleeding (95), (166). The pace of recovery depends, within limits, on the amount of hemoglobin available. Not only are cells of better quality put out when this is abundant, but there then occurs an extension of the red marrow, so that soon the body comes to possess more blood-forming tissue with which to meet the situation (122).

The prevailing view on the normal fate of hemoglobin is that it is broken up into globin and hematin, which latter in turn yields hematoidin,—thrown off in the bile as bilirubin,—and iron-containing materials of uncertain chemical constitution which are retained (1), (235). When an unusual amount of the iron-containing stuff collects in the body it can often be recognized by microchemical reactions. Recent work makes one doubt whether that is all there is to the matter. Two sorts of bilirubin have now been distinguished, one formed through liver activity, and another arising without intervention of the organ (220), (221); and certain suggestive anomalies of pigment metabolism have been described. There is a condition of congenital origin in which hematoporphyrin (78), nearly related to hematin, is excreted in the urine in large amount, and in which, moreover,hematin itself is found in the blood, together with bilirubin (196), (197). In some diseases iron has been found in the plasma in such a state that it may be readily demonstrated, whereas in others it occurs in "masked form" (220). Hematin (194), (68) may circulate in such quantity as to color the plasma a yellow-brown (194). The obdurate character of this pigment had led to the supposition that it cannot be an intermediate stage in the normal

degradation of hemoglobin (33), (34), (35), (36). The truth would seem to be that at present one can only make conjectures about the course normally followed in the degradation of hemoglobin. The routes for its destruction under pathological circumstances are many-tracked. The relation of the hemoglobin of the blood to myohematin, to the hemofuscin of hemochromatosis, to the pigment of brown atrophy, and to the yellow or brown material laid down in certain organs as the body ages, has all to be worked out, as has the connection of the blood pigment with the urinary pigments and with the iron normal to the cells of the tissues in general (80), (19), (4).

Repeated attempts have been made to work backwards up the trail of the hemoglobin derivatives to the sites, if not of blood destruction, then of the pigment metabolism. The results of analyses of the organs for iron have given rise to interesting and diverse hypotheses on the fate of the erythrocyte (4), (191), (115). But numerous facts point to a need for caution in this connection. The bone-marrow of normal man, a tissue not greatly concerned, so far as is known, in the destruction of corpuscles, yields the microchemical reactions for iron more often than does any other tissue (191). The iron derived from hemolyzed corpuscles is not distributed like that from phagocyted ones (141). Furthermore, local injury without blood extravasation may result, under appropriate conditions, in a local accumulation of hemosiderin (179). And in hemochromatosis, a disease characterized by the extensive deposition of iron-containing pigments, there is no evidence whatever of increased blood-destruction but much that is practically conclusive against it (131), (205), (92). The peculiar abundance of hemosiderin in the hepatic parenchyma of patients with pernicious anemia can scarcely attest, as so many think, to a portal disintegration of erythrocytes. For the repeated injection of small quantities of hemoglobin into the systemic circulation will bring about a like condition (123). And how is one to reconcile the idea of a "physiological extravasation" of blood in the spleen during the course of pernicious anemia with the fact that the iron content of the organ proves frequently to be less than normal (194)? All this, and more, tends to weaken inferences from iron-distribution. Nevertheless, it may serve to confirm impressions gained by other methods. For example, the iron content of the spleen and liver is such as to support the belief that these organs are important in the conservation of blood pigment. The ferruginous material freed and stored during experimental blood destruction is drawn upon in the subsequent repair (142).

COMMENT

Few generalizations can be gleaned at the present time from the confused multitude of observations upon blood destruction. But this much at least is sure: A disintegration of red corpuscles takes place constantly, in sickness and health, and the body has often to meet new demands and situations connected therewith. These are usually coped with successfully, in so far as concerns the immediate task of accomplishing additional destruction, when that is necessary, or disposing of the products of a breaking-down unprovoked by the body. But for such emergency purposes other mechanisms besides the normal must frequently be called upon. It is the interplay and substitute activities of these reserve mechanisms which make conclusions so difficult upon the normal as well as the pathological course of events. Given the many heterogeneous phenomena of blood destruction, representing reactions of the body to disease and to experimental procedures, the task of the investigator is to determine the precise elements responsible for such physiological combinations and permutations. And it is not as if the elements concerned had fixed dimensions or values. When one is excluded from a rôle in the organism, others assume an unwonted importance and the net result is the same in all save details of execution.

The reader who has come thus far will not fail to have noted that much of current theory upon blood destruction attests to the theorist's abhorrence of a vacuum. If the reviewer, in discussing various conceptions has seemed to blow both hot and cold as regards them, this is no more than the facts themselves do. And yet the profusion and diversity of these facts already gained is such that they will surely suffice to embody and extra-illustrate many times over the complex principles regulating the fate of the red cell,—once these principles stand clear.

Note on the bibliography. Much of the literature on blood destruction is shut away behind titles and within subjects that a reviewer will naturally pass by. In that here cited less regard has been had for priority, and perhaps even for significance, than for aptness in illustration, and usefulness to the enquirer. A selection, and in the nature of the case a relatively small selection, has been made from the great mass of titles.

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THE ETIOLOGY OF RICKETS

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"The history of rickets is that it has been enriched by a wealth of new hypotheses but few new facts."[1] This statement made in 1908 is no longer true since in the past four years facts of the greatest value have been discovered.

In 1650 Glisson (2) established rickets as a clinical entity, which received yet sharper definition, in the latter part of the last century, as cretinism, chondrodystrophy, osteogenesis imperfecta and scurvy were disentwined from the supporting stem through the work of Virchow, Parrot, Porak, Kirchberg, Marchand, Stelling, Kaufmann, Barlow and others. In 1885 Pommer (3) established on a firm foundation for all time the pathology of rickets through magnificent histological studies which were corroborated and extended by v. Recklinghausen (4), Schmidt (5) and Schmorl (6). The past four years have been marked by inroads into the etiology of rickets, and the past two years by the beginnings of an insight into the disturbance of the salt metabolism which constitutes the disease.

Rickets is a disturbance in the metabolism of the growing organism of such nature that the salt equilibrium, in particular as regards the calcium and phosphorus, in the circulating fluids is disturbed, and lime salts no longer deposit in the bones. Lime salts may not deposit because the ionized calcium in the blood is low, or because the ionized phosphate is low, or because both are low. When, however, the calcium in the blood is low, the formation of new bone and the destruction of old calcified bone (umbau) is greatly accelerated, and the pathological process takes on a distinctive character. But no fundamental differences exist between the low calcium and the low phosphorus forms of the disease. Increasing knowledge concerning rickets has made it necessary to broaden the view in regard to the characteristic pathology and admit to the disease all disturbances in metabolism in which lime salts cease to be deposited in the bones and cartilage. The first

[1] Quotation from Albu and Neuberg cited by Findlay, Paton and Sharpe (1).

detectable signs of rickets are probably a diminution of the inorganic phosphorus or calcium of the blood.

THE OCCURRENCE OF RICKETS: *The geographical distribution.* For a complete presentation of this subject the reader is referred to the splendid article by Palm (7) and to the recent book of Dick (8), and to the historical sketch by Findlay (9) and to the book by Dick for the evidence concerning the existence and distribution of rickets in ancient times. Here one should mention a few facts. Rickets occurs chiefly in Europe and North America. It is a disease found in cities. It is most prevalent in those nations whose wealth and industrial development have brought about most fully the substitution of artificial conditions of living in place of the simple conditions which nature intended. Wherever civilization of this artificial character establishes new contacts, rickets begins to appear. The disease has appeared in India where the designs of nature have been interfered with by religious customs to be mentioned later. The disease never occurs among peoples living under natural conditions. Savages may starve and may become the victims of pestilence, but they do not develop rickets. The disease does not occur in the native parts of Africa and is exceedingly rare in China and Japan. It occurs rarely in the tropics, and is rare in the Arctic regions.

The prevalence among animals. Jost and Koch (10) state that rickets is a common disease among pigs, puppies, lambs and kids, but less common among colts, calves and rabbits. It manifests itself with comparative frequency among carnivorous animals and also among monkeys in captivity. The striking facts concerning the occurrence of rickets among animals are as follows: The disease appears only among those animals which man has been able to make captives or slaves and upon which he has been able to impose successfully such artificial conditions of environment and diet as he has developed for himself in his struggle for existence. The disease never develops among animals living apart from man and probably cannot develop in animals or in man in the wild state. Hansemann (11) points out that the cat, in contrast to the dog, never develops rickets. He gives as his reason the fact that the cat, though tamed, can never be made to relinquish the habits natural to the species. Apparently rickets develops frequently in the monkey when he is in the zoölogical garden, but never when he is at liberty. Rickets can be produced in the domesticated rat but only by unusual means. It is probable that the rat is not readily susceptible to the development of rickets.

The frequency of occurrence. Inasmuch as rickets can be determined by clinical means in only a fraction of the cases, it is impossible to obtain exact information concerning its frequency of occurrence. One can say that rickets is so common in the large cities of America and Europe that few children among the poorer classes are untouched by it. Data bearing on this subject will be found in the section devoted to the seasonal variation in rickets.

The occurrence in utero. For a complete discussion of the controversy concerning this question, the reader is referred to Wieland (12). The most violent advocate of the congenital origin of rickets in recent times has been Kassowitz (13). He believed that rickets had a fetal origin in a large percentage of cases. The clinical evidence consisted in soft areas in the bones of the head, enlargement of the fontanelles and of the lines of suture, prominence of the bosses, enlargement of the costo-chondral junctions, and protuberance of the abdomen. The anatomical evidence need not be recited, for Kassowitz's understanding of the pathological changes and processes in rickets was such as to lead him to confuse the normal condition with the rachitic, and to make him unable to separate rickets from syphilis.

The best evidence against congenital rickets has been brought forward by Schmorl (6) who investigated the ribs and long bones of the extremities of more than 100 infants born either at term or prematurely, without once finding evidences of the disease. Pommer (3), Lentz (14), Tschistowitsch (15) and Wieland (12) have shown conclusively that the spots of defective ossification in the skull in new-born infants have no connection with rickets.

The only way in which proof of the existence of congenital rickets can be obtained is by histological examination of the bones. Whatever reliable evidence comes from that source indicates that rickets does not occur in utero. Until further studies of the bones of new-born children are made, it is probably unsafe to conclude that rickets can never develop in intra-uterine life. One may be certain, however, that if rickets ever occurs in intra-uterine life it does so rarely, and it never exceeds the slightest degree of development.

The earliest age at which rickets occurs. It is commonly taught that rickets first begins about the third or fourth month, because the first clinical signs of the disease, head sweating and craniotabes, make their appearance about then. When, however, clinical evidences of the disease exist, the pathological changes in the bones have become fairly advanced, and the disturbance in metabolism which gives rise to them must have

been in existence for some time. Anatomical studies of large numbers of children, from the time of birth to four years of age, have been made by Schmorl (6) with a view to determining the age incidence of rickets. The earliest age at which Schmorl was able to find evidence of the disease was one and one-half months. In this clinic, however, Dr. Ethel Dunham[2] has discovered well-advanced rickets in a prematurely born infant aged only one month. Both Yllpö (16) and Hamilton (17) have commented on the fact that rickets makes its appearance earlier in prematurely born infants than in those born at term.

If rickets can become sufficiently advanced to manifest itself at the age of one month in lesions which are easily demonstrable by the x-ray at the ends of the long bones of the extremities, it is clear that the disease commenced immediately after birth. Doubtless rickets actually begins in the case of many children immediately after birth. Indeed, such cases as the one discovered by Doctor Dunham make one hesitate to think that rickets cannot begin in intra-uterine life.

The frequency in prematurely-born infants. Both rickets and tetany are exceedingly prevalent in infants born before term. According to Rosenstern (18), 76 per cent of all premature infants exhibit evidences of tetany. The statistics of Yllpö (16) indicate that 35 per cent of premature infants have tetany. As is obvious, no one can say how frequently rickets occurs in infants whether prematurely born or at term. It is certain, however, that the great majority of premature infants, in the countries in which rickets is common, develop the disease whether suckled or fed artificially.

It is interesting that both tetany and rickets are apt to manifest themselves differently in premature infants than in those born at term. The tetany appears early, is apt to be latent and becomes manifest only in the presence of infection. It tends to disappear naturally about the sixth month if breast milk feeding is continued. The rickets shows itself early, as has already been pointed out, and is characterized by deformities of the head which are out of proportion to those of the extremities, a fact of general knowledge emphasized recently by Hutchison (19) and Yllpö (16).

The seasonal variation. The observation that there is a seasonal variation in the incidence of rickets and tetany was made years ago and has been remade many times since.

In 1884 Kassowitz (20) commented at length on the rise in the curves of incidence of rickets and tetany during the winter months and their

[2] The report of this case is to be published.

decline during the summer and autumn, and he used the parallelism between them as an argument for the unity of tetany and rickets.

The seasonal variation in rickets was beautifully demonstrated in the pathological studies of Schmorl (6). These studies showed that rickets may begin at any time, but the highest percentage of early manifestations of the disease is between November and May. The percentage of cases with signs of healing increased as the summer progressed and reached its highest point in the autumn, only to fall again as the winter months came.

Hansemann (11) noted that almost all children who are born in the fall and die in the spring show marked manifestations of rickets, in contrast to the children born in the spring and dying in the fall who are almost free from rickets.

In this country Hess (21) has emphasized the importance of the seasonal variation in rickets, and recently Lundagen and he (22) have reported a seasonal variation in the level of the inorganic phosphate of the blood (270 determinations; the number of children investigated not stated). The average figures for the inorganic phosphate of the blood serum were: June and July, 1921, 4.35 mgm. per cent; December, 3.92 mgm.; January, 1922, 3.9 mgm.; February, 3.76 mgm.; March, 3.58 mgm.; April, 3.68 mgm.; May, 3.98 mgm. The authors conclude that there is a seasonal variation in the level of the inorganic phosphate of the blood of the normal infant which corresponds to and is determined by the seasonal variation in the richness of the solar spectrum in ultra-violet rays. Of course, in order to be sure that a seasonal variation in the blood phosphate is a normal phenomenon in the infant, it would be necessary to examine repeatedly during a year great numbers of infants and children living at widely separated places and under the most varied conditions. Unfortunately, in the paper by Hess and Lundagen data on this point are lacking. It seems most improbable, however, that slight seasonal differences in the level of the inorganic phosphate of the blood found by Hess and Lundagen are to be interpreted as meaning that a "seasonal tide of the blood phosphate" is a physiological phenomenon; but rather that at the time of the year when the incidence of rickets is highest the disease in its mildest form is almost universal under the conditions of hygiene and diet which surround infants in cities, in particular those living in institutions. The infants studied by Hess and Lundagen were probably living under conditions commonly accepted as normal for the infant but were not those which nature intended. We know already, from the

work of Schmorl and others, how extraordinarily widespread rickets is among the infants and children of cities. Schmorl (6) found that 90 per cent of the children in Dresden under four years of age who died between the years 1901 and 1908 and more than 96 per cent of the infants showed evidences of rickets, and Hess (23) himself has reported that rickets was exceedingly prevalent among the children in the Hebrew Infant Asylum in New York City, where presumably the chemical investigations of the blood were made. The investigations of Hess and Lundagen, then, are probably to be regarded as further corroborations of the seasonal variation of rickets and of the universality of rickets among infants and children living under the conditions which prevail in our large cities.

The association with other diseases. Rickets frequently accompanies infantile scurvy; indeed, it is exceedingly uncommon to find scurvy uncomplicated by rickets. The association of rickets and tetany is also exceedingly close. A very large proportion of children with rickets show also tetany, as has been pointed out by Kassowitz (20) and many others, and recently by Ferguson (24).

THEORIES CONCERNING THE CAUSE OF RICKETS: *Several hypotheses that need only be mentioned.* Glisson (2) believed that rickets was the result of over-eating (over-nutrition) (8) and recently Jundell (25) has revived this idea. Heitzmann (26) proposed that the disease was caused by an acidosis which brought about a decalcification of the bones, a hypothesis which has been advanced recently by Klose and Vogt (27) in explanation of the rickets which their thymectomized dogs developed and which has been advocated also by Pritchard (28). Several French clinicians (29) have held that rickets is a manifestation of congenital syphilis or have attributed it to auto-intoxication. The theories that rickets is the result of infection or is inherited or is due to the disturbance in the functions of the organs of internal secretion, will be considered in that portion of the paper which immediately follows. The two most important theories, namely, that rickets is due to deprivation of light or is caused by defective diets, will be discussed at length in the remainder of the paper.

Is rickets inherited? Siegert (30) is the exponent of the theory of inheritance. He made statistical studies of thirty-one families in which the children were breast-fed but had rickets, and of fourteen families in which the children were breast-fed but were free from rickets. In twenty-nine of the families of the first group he found that the mother either had deformities of rickets or gave a history of rickets, and in the remaining

two families he discovered evidence which made the previous existence of rickets in the mother probable. In the second group, in which the children were free from the disease, the parents also were free. When he investigated families in which the rachitic children were artificially fed, the incidence of rickets in the parents was not so striking. In the main, however, Siegert found that one or both parents had been sufferers from rickets if the children had the disease. Siegert concluded that heredity is the most important factor in the etiology of rickets and that the disease is most commonly transmitted through the mother, though he brought forward some evidence to indicate that it might be transmitted through the father. If both parents were rachitic, he thought rickets in the offspring was inevitable. Siegert was greatly puzzled on finding some children in the rachitic families who had escaped the disease. It is an amusing sidelight on the subconscious workings of the mind of an investigator over-intent on his theory, that Siegert should have thought such children were probably illegitimate. The family history of rickets might be taken as supporting the theory that heredity bears a part in its cause. It might also be taken as evidence that rickets is of the nature of an infection, or it may simply mean that the habits and ways of living of one generation have been continued in the next. Inasmuch as rickets consists in a disturbance of metabolism, however, a predisposition may be inherited. Pigmented skins apparently increase the susceptibility to rickets, (31) and a predisposition in that sense may be inherited. The disease, however, cannot be inherited through the germ plasm.

Is rickets the result of a disturbance in the function of the organs of internal secretion? Rickets has been ascribed to disturbances in the function of the thyroid, parathyroid, thymus and adrenal glands, and the carotid bodies. Lanz (32) suggested the use of the thyroid gland in rickets, but Knoepfelmacher (33) and Heubner (34) tried it without success. Stoeltzner (35) announced favorable results from the use of the adrenal gland, but other investigators were unable to corroborate his findings, and finally even Stoeltzner is said to have abandoned the idea (30). A large number of investigators have reported that the removal of the thymus caused rickets. Chief among them may be mentioned v. Basch (36), Klose and Vogt (27) and Matti (37). Their work has been challenged and overturned, however, by Nordmann (38), Pappenheimer (39), Renton and Robertson (40) and McClure and the writer (41) of this paper. Erdheim (42) has reported that rickets follows removal of the parathyroid glands. Both Erdheim (42) and

Pappenheimer (43) have found the parathyroid gland to be hypertrophied in rickets. It is probable, however, but not certain that the function of the parathyroid glands has no causative relationship to the development of rickets. The *reductio ad absurdum* of the theories of interrelationship between the function of the organs of internal secretion and the development of rickets is to be found in the work of Betke (44). This investigator reported that removal of the carotid bodies was followed by the development of rickets and the same elaborate train of symptoms which Klose and Vogt described after removal of the thymus.

While it is conceivable that the vitamines or radiant energy are connected in some way with the functioning power of the organs of internal secretion, it seems most probable that the rickets which developed in animals after removal of the organs of internal secretion was due to other causes. Pappenheimer encountered rickets both in his thymectomized rats and in his control rats. Similar experiences are not at all uncommon in the case of animals in laboratories.

Is rickets a result of infection? Iovane and Forte (45) and Moussu (46) advocated the infectious origin of rickets. Their experiments, however, had little value.

Morpurgo (47) in 1898 noted the spontaneous appearance of a disease resembling rickets and osteomalacia in a colony of white rats of stock which had been in the laboratory for seven years. He obtained a diplococcus from the infected animals, inoculated other animals with it, and found that they developed the disease. Morpurgo supplies no information concerning the food which the rats received. The organism was not recovered from any of the inoculated rats.

Koch (48) also reported the production of rickets by means of inoculation with bacteria. Altogether Koch inoculated seventy dogs, and had more than one hundred control dogs. After preliminary experiments he chose the streptococcus longus as the organism most likely to produce rickets. During the first three or four days the dogs developed swollen joints and showed symptoms which were evidently the result of infection. In typical instances a period then followed during which the animals seemed well. Before long, however, rachitic deformities appeared and reached an advanced development. The dogs were kept in horse stalls and were fed milk, semmel, meat and potatoes, with the addition of salt. Koch states that three of thirty control dogs also developed rickets. Koch interpreted his experiments as meaning that rickets is produced by an organism having a special

affinity for the skeleton. The arthritis and constitutional symptoms marked the invasion of the skeleton, and represented the acute stage of the disease; the rachitic deformities were the results of the inflammation after it had become chronic.

Years ago Edlefsen (49) favored the infectious origin of rickets as the result of observations on children. He thought the enlargement of the spleen in rickets significant and regarded fever as an early manifestation. The recurrence of rickets in certain houses suggested that the houses harbored some infectious agent.

A careful search of the literature would bring to light the names of many exponents of the infectious origin of rickets. Clinical experience and investigation, however, have made clear that infection cannot possibly be more than a contributory factor.

THE EXPERIMENTAL PRODUCTION OF RICKETS[3] IN ANIMALS AND THE KNOWLEDGE DERIVED THEREFROM. Sixteen years ago Hopkins (50) wrote: "But, further, no animal can live upon a mixture of pure protein, fat, and carbohydrate, and even when the necessary inorganic material is carefully supplied the animal still cannot flourish. The animal body is adjusted to live either upon plant tissues or the tissues of other animals, and these contain countless substances other than the proteins, carbohydrates, and fats. Physiological evolution, I believe, has made some of these well-nigh as essential as are the basal constituents of diet. Lecithin, for instance, has been repeatedly shown to have a marked influence upon nutrition, and this just happens to be something already familiar, and a substance that happens to have been tried. The field is almost unexplored; only is it certain that there are many minor factors in all diets, of which the body takes account. In diseases such as rickets, and particularly in scurvy, we have had for long years knowledge of a dietetic factor; but though we know how to benefit these conditions empirically, the real errors in the diet are to this day quite obscure" (p. 395). In the book entitled "Die Vitamine" by Funk (51), published in 1914, the following sentence appears: "It is very probable that rickets occurs only when certain substances in the diet essential for normal metabolism are lacking or supplied in

[3] Voit, Dibbelt, Aron and Sebauer, and Stolzner and Miwa have attempted to produce rickets in animals through the administration of diets deficient in calcium. Their experiments took place before the importance of an exact understanding of the composition of the diet was appreciated, and before the pathology of rickets had become generally understood. Although their work contains much information of great value, no presentation of it will be made in this paper.

insufficient amount. The substances occur in good breast-milk, also in cod liver oil, but are lacking in sterilized milk and in cereals" (p. 127). Funk had reference to the so-called "vitamines." When, accordingly, Mellanby (52) made the announcement in 1918 of the successful production of rickets by means of a diet lacking in "an accessory factor," medical opinion was in a measure prepared. The report of his first experiments was meagre and would probably have awakened little interest, had not the British Medical Research Committee endorsed the work and publicly committed itself to the view that rickets is a deficiency disease due to a lack in the diet of an "anti-rachitic factor" (53).

Although the experiments of Findlay (54) preceded those of Mellanby by ten years, it was the work of Mellanby which gave to the investigation of rickets its impetus. For this reason and also because the more recent experiments of Findlay, Paton and their co-workers seem to have been designed to attack the dietetic theory of rickets, the experiments of Mellanby will be described before those of the Glasgow School.

The experiments of Mellanby. Mellanby's experiments (55) were performed entirely on puppies and reached a total of almost four hundred. The animals were about two months old when the experiments were begun, and were kept on the test diets for a number of weeks or months until rickets had developed or had failed to develop. The diet used by Mellanby as a standard for the production of rickets in the puppies underwent a natural evolution as may be seen from the following table:

Rachitic diets

DIET I	DIET II	DIET III	DIET IV
Whole milk, 175 cc.	Whole milk, 175 cc.	Separated milk, 175 cc.	Separated milk, 250–350 cc.
Oatmeal, rice.	Bread ad lib.	Bread (70 per cent wheaten) ad lib.	Bread (70 per cent wheaten) ad lib.
1–2 gm. NaCl.		Linseed oil, 10 cc.	Linseed oil, 5–15 cc.
		Yeast, 10 gm.	Yeast, 5–10 gm.
		NaCl, 1–2 gm.	Orange juice, 3 cc.
			NaCl, 1–2 gm.

The principle on which Mellanby proceeded was as follows: Having established a standard diet which regularly produced rickets in the puppy, he introduced into it in turn food substances whose influence

for or against rickets he desired to determine. For example, in experiment 282 he substituted 10 grams of lard for the linseed oil. The development of rickets in the puppy was uninfluenced. Mellanby was then able to infer that lard contained no substance of an anti-rachitic nature. The great majority of Mellanby's dogs were confined indoors. In a few experiments, however, in particular in some of those in which the influence of confinement on the development of rickets was under investigation, the animals were either confined out-of-doors or allowed a run in an enclosure. One assumes that the majority of his dogs lived in roomlight (daylight filtered through ordinary window glass). There is every reason to suppose that his animals received excellent care. The dogs under observation seem to have been abundantly controlled, a point of great importance, though the control animals were not from the same litters.

The criteria which Mellanby used to determine whether a puppy had or had not rickets were: *a*, the appearance; *b*, the x-ray findings; *c*, the determination of the calcium oxide in the bones; and *d*, histological study of the bones. The reader gains the impression that Mellanby himself had no special knowledge of the histological changes characteristic of rickets, and that he depended largely on x-ray examination and on the determination of calcium oxide.

Some very interesting results of Mellanby's experiments are the following: Among the oils, cod liver oil was found to stand alone in its anti-rachitic potency; suet and butter fat exerted a strong influence on calcification, while lard had no anti-rachitic effect; of the vegetable oils, peanut oil stood first, then cocoanut oil; rapeseed, cottonseed, palm kernel, olive and linseed oils produced little or no effect; babassu oil was the "worst;" cod liver oil was found to be superior to butter fat and invariably prevented rickets; butter fat did not always prevent rickets. Mellanby (55) reached the very interesting conclusion that "butter is a more potent anti-rachitic agent when it has abundance of calcium salts, as found in milk, with which to work. It is but natural that the anti-rachitic vitamine of butter, which certainly has a strong influence on the deposition of calcium phosphate in bone, should also have a sufficiency of calcium salts in the diet before it can work effectively" (p. 53).

Mellanby performed a most interesting experiment which has since been repeated with much greater success by McCollum, Simmonds, Becker and Shipley (56). Hopkins (57) had shown that when the temperature of butter fat or cod liver oil is maintained at 120°C. for four

hours in a stream of oxygen, fat-soluble A is destroyed. Mellanby fed butter and cod liver oil, oxidized in this way by Hopkins himself, to puppies on the standard diet. The puppy receiving from 5 to 10 grams of oxidized butter was not protected from rickets, whereas the puppy receiving the untreated butter was protected. In contrast, the puppy receiving 10 cc. of oxidized cod liver oil was completely protected from rickets. If Mellanby had made certain that the oxidized cod liver oil was devoid of fat-soluble A, he would have known that the substance in cod liver oil which prevented rickets was not fat-soluble A.

Mellanby found that lean meat has an anti-rachitic action which seemed, however, to be of a different order from that of the vitamine-containing fats. Meat, he observed, made the puppies eat better and, under favorable experimental conditions, prevented or limited the extent of the rickets; under unfavorable conditions it did not prevent the development of rickets. Bread seemed to favor the development of rickets. The more bread eaten, the more marked the rickets. Casein retaining its calcium (alkaline casein) prevented the development of rickets; casein from which the calcium had been removed by treatment with hydrochloric acid (acid casein) seemed to intensify the rickets-producing power of the diet. The feeding of thyroid gland did not inhibit the development of rickets. Some animals, in particular those receiving the purified casein, showed signs of tetany.

Some of Mellanby's general observations are interesting. If the diet was well constituted, a small amount of the "anti-rachitic vitamine" appeared to suffice; if badly constituted, in a rachitic sense, a large amount was necessary. The older the animal, the more difficult the production of rickets and the more independent of the "anti-rachitic vitamine" the animal seemed to be. Mellanby observed that "osteoporosis" in dogs may give rise to deformities identical with those of rickets. The addition of tricalcium phosphate to the standard diet did not prevent rickets.

The view of Mellanby concerning the causation of rickets was broad. Though holding rickets to be a deficiency disease due to the absence of an "anti-rachitic vitamine" in the diet, he believed that defective environmental conditions and confinement contributed to its development.

The experiments of Mellanby were open to obvious criticism: his diets were more crude than those of some other investigators; the salt composition of the diets was not taken into consideration; good histolog-

ical studies of the bones and chemical examinations of the blood were lacking; the criteria on which chief reliance was placed were not satisfactory. Nevertheless, the experiments of Mellanby constitute pioneer work and as such were splendid. To him belongs the credit of establishing the relationship between the development of rickets and the deficiency in the diet of a vitamine.

Experiments of Findlay and the Glasgow school of investigators. In 1908 Findlay (54) published his well-known experiments on the production of rickets by confinement. Led by the idea that rickets was a dietetic disorder, he first attempted to produce it by feeding diets composed of cereals or bread and water, but found that the puppies became marantic and did not develop rickets. Control puppies, however, fed a diet of milk and porridge, did develop rickets, with the exception of one animal which, as it happened, was exercised daily in the open air. In this way Findlay's attention became attracted to the possibility of a connection between rickets and muscular activity. Findlay renewed his experiments. The test animals were kept in cages in the laboratory, while the control animals were allowed to run about the floor. The diet was milk and porridge. Eight confined puppies developed the disease, whereas the unconfined puppies remained free. Findlay stated with great positiveness that rickets ". . . . . is due to the want of exercise which invariably goes along with it or is consequent on confinement" and concluded with the sentence: "Alike, then, on clinical and experimental grounds, I accordingly conclude that confinement, with consequent lack of exercise, is the main factor in causing the disease."

The diet used by Findlay was poor and similar to the one employed subsequently by Mellanby for the production of rickets in puppies. Good control experiments amounted only to five. No doubt exists that Findlay's puppies had rickets.

The stir created by Mellanby's work had already become apparent, for in 1918 Paton, Findlay and Watson (58) reported experiments which seem to have been planned to put Mellanby's theories and theirs to a comparative test; and in 1921 Paton and Watson (59) made an actual foray into Mellanby's domain. Space does not permit a description of these interesting but rather complicated experiments of the Glasgow investigators. It is sufficient to say that the experiments indicated: dogs allowed a run in the country do not develop rickets; butter fat does not prevent rickets, but possilby exerts a slight protective action; high calorie diets rich in whole milk seem to exercise some protective influence; confinement alone does not cause rickets.

Rickets cannot be due to "confinement with lack of muscular exercise." The idea that the primary cause of rickets lies in the inability of the child to gratify a natural impulse for exercise is difficult to accept on *a priori* grounds. Some years ago Howland and the writer[4] of this paper, and, later, Baldwin[4] confined puppies in small cages for two or three months, but could not obtain rickets in that way. Mellanby also showed clearly that confined puppies will not develop rickets if the diet is properly constituted. Though Paton and Watson failed to emphasize the fact that their experiments did not turn out in accordance with the theory of confinement, both they and Findlay must have been conscious of the fact, for in 1921 Paton showed signs of retiring upon the hypothesis that rickets is due to infection (59), and Findlay has recently withdrawn to a new position where nature has erected an unassailable stronghold,—sunlight. In his most recent paper on the subject of rickets Findlay (60) has written: ". . . . defective hygiene, using this term in its widest sense, is the most important known determining factor in the causation of rickets" (p. 826).

The experiments of American investigators. Almost simultaneously Sherman and Pappenheimer and McCollum, Simmonds, Shipley and the present writer published papers reporting the production of rickets in the rat by means of diets having specific defects in their salt composition.

In January, 1921, McCollum, Simmonds, Shipley and the present writer (61) reported that a number of different diets produced abnormal conditions in the skeleton of the rat which corresponded closely to or appeared actually to be identical with the rickets of the human being. All of these diets were known to be defective in fat-soluble A. One was defective in its phosphorus content, though the significance of this was not appreciated at the time; the others were defective in calcium. In general the proteins used were of poor quality. The investigators had before them at that time what subsequent investigation revealed to be two kinds of rickets, one characterized by a calcium phosphorus ratio in the blood which was low, the other by one which was high. Conditions appeared to be too complicated to permit of any single explanation. The writers, therefore, stated: "At present it is only possible to say that the etiological factor is to be found in an improper dietetic regimen. The large number of dietary formulae, the administration of which results in rickets and kindred affections,

[4] These experiments were never reported.

gives abundant evidence of the complex nature of the causes operating in the production of the disease— . . . ." (p. 340).

In March, 1921, Sherman and Pappenheimer (62) published a most important paper which described the production of rickets in the rat by means of a diet low in phosphorus and its prevention by means of the addition to the diet of alkaline potassium phosphate. The diet was composed of patent flour, 95 per cent, calcium lactate, 3 per cent, sodium chloride, 2 per cent, with or without the addition or a trace of ferric citrate; this regularly produced rickets which possessed all the characteristics of the disease in the human being. When alkaline potassium phosphate was added to the diet in the proportion of 0.4 per cent, and an equivalent amount of calcium lactate was withdrawn, the animals were completely protected from the development of rickets. The diet which Sherman and Pappenheimer used was deficient not only in fat-soluble A and phosphate but in water-soluble B, water-soluble C, protein and potassium. Accordingly, it was impossible to be certain that the rickets occurred solely as the result of the deficiency in the phosphorus. In publications which soon appeared (63), (64), (65), however, Pappenheimer, McCann, Zucker and Hess showed that the additions to the diet of yeast in small amounts, orange juice, butter fat in moderate amount, and potassium did not exert the inhibitory influence.

The paper of Sherman and Pappenheimer was soon followed (May) by the publication of kindred experiments by Shipley, McCollum, Simmonds and the present writer (66). These investigators reported the production of rickets by means of two diets. One was composed of rolled oats 40 per cent, flaxseed meal 8.3 per cent, sodium chloride 1.0 per cent, calcium carbonate 1.4 per cent, dextrin 49.2 per cent. The other was composed of rolled oats 40.0 per cent, gelatin 10.0 per cent, sodium chloride 1.0 per cent, calcium carbonate 1.5 per cent, and dextrin 47.5 per cent. Both of these diets were known to be deficient only in fat-soluble A and in phosphorus, and in other respects were well constituted. When a complete salt mixture was substituted for the inorganic constituents of diet 2806, so that the deficiency in phosphorus was removed, the diet no longer produced rickets, but typical osteoporosis.

The experiments of Sherman and Pappenheimer and McCollum, Simmonds, Shipley and the present writer marked a distinct advance in the investigation of rickets in several respects, especially because they showed the importance of the inorganic constitution of the

diet in particular of the phosphorus, in relation to the disease. With the exception of Mellanby, who had placed but little importance on the salt composition of the diets, other investigators had attempted to produce rickets by means of reduction of the calcium.

Since the subsequent experiments of Pappenheimer and those associated with him, Hess, Zucker, McCann and Steiner, and the experiments of McCollum, Simmonds, Shipley and the present writer showed a remarkable parallelism and led to almost identical conclusions, they will not be treated separately.

Cod liver oil cures rickets and prevents its development. According to Trousseau, cod liver oil has been used from time immemorial as a folk-remedy on the coast of England, Holland and France. It was introduced into France as a specific for rickets by Bretonneau, and its uses became general through the teachings of Trousseau. Trousseau himself was convinced of its curative action not only in rickets but also in osteomalacia and many physicians have been certain of its effectiveness in rickets solely as the result of clinical observation. Schabad (128), Orgler (122) and Freund (130) have brought forward indirect evidence of the curative action of cod liver oil in rickets by means of metabolism experiments which will be referred to later. The direct proof of its curative action, however, was first obtained by McCollum, Simmonds, Shipley and the present writer (74). These investigators discovered early that cod liver oil caused remarkable depositions of lime salts to form in the cartilage of the rachitic rat close to its junction with the metaphysis (the so-called "line test"). Howland and the present writer (139) proved by means of the x-ray that the administration of cod liver oil to rachitic children was followed by the deposition of lime salts in the cartilage and bones after a period of fifteen to twenty-one days.

The relationship of fat-soluble A to the development of rickets. Diets deficient in fat-soluble A alone do not produce rickets. This fact was first clearly proved in the experiments of Sherman and Pappenheimer (62) and in those of Shipley, McCollum, Simmonds and the present writer (66) already described. The group of investigators last mentioned clearly stated that "a deficiency in fat-soluble A cannot be the sole cause of rickets," (p. 165) and that the pathological condition induced by diets deficient in fat-soluble A alone was "osteoporosis." The subsequent experiments of Pappenheimer and the investigators associated with him (67) and of McCollum, Simmonds, Shipley and the present writer (68) have repeatedly attested to the truth of these observations. Zilva, Golding, Drummond and Coward (69) Mackay (70) and Tozer (71)

also came to similar conclusions as the result of experiments on the pig, kitten and guinea pig. There can be no doubt that in man a deficiency in the diet of fat-soluble A alone does not give rise to rickets. Indeed, there is evidence to show that fat-soluble A in the diet may be necessary for the full development of the rachitic process, as will be pointed out later.

The organic substance or factor in cod liver oil which causes lime salts to deposit in the bones in rickets is in all probability distinct from fat-soluble A. (In order to avoid constant repetition, it will hereafter be termed *X*. By *X* is understood that substance or group of substances, factor or factors so richly contained in cod liver oil, which exert the regulatory influence on the metabolism in rickets and bring about the deposition of lime salts in the bones.)

Believers in the dietetic origin of rickets were in a dilemma. The investigators working with rats had shown that rickets could be made to develop by means of diets defective in their salt composition, but not if at the same time the animal was receiving cod liver oil. Cod liver oil obviously contained some substance inhibitory to the development of rickets. Yet in the absence of cod liver oil from the diet, rickets would not result if the salt defect was removed by the addition of phosphate or by the substitution of a well-balanced salt mixture. Obviously the development of rickets in the rat depended, other things being favorable, not on a single contingency but on two contingencies: *a*, the absence from the diet of an organic substance contained in cod liver oil which, so to speak, threw open the door; and *b*, a disproportionate relation in the inorganic constituents of the diet which, so to speak, caused rickets to enter. Now cod liver oil was especially rich in fat-soluble A. The natural assumption was, therefore, that the inhibitory organic substance in cod liver oil was fat-soluble A. Butter fat, however, also rich in fat-soluble A, exerted an exceedingly feeble action. No advocate of the dietetic theory of rickets could deny, on the one hand, that an organic factor was concerned in the development of the disease, or, on the other hand, dared say that an organic factor distinct from fat-soluble A was concerned.

In July, 1921, McCollum, Simmonds, Shipley, and the present writer, influenced by the differences in the effectiveness of butter fat and cod liver oil when added to the diets of rats in a state of calcium starvation, suggested the existence of a dietary essential in cod liver oil separate from fat-soluble A (72). As the result of further experiments on the rat with diets containing butter fat and cod liver oil, the investigators just

named were able to write, in January, 1922, the following statement (73): "The results of this series of experiments were so consistent and decisive that we can deduce no other conclusion than that cod liver oil contains in abundance some substance which is present in butter fat in but very slight amounts, and which exerts a direct influence on the bone development, and enables animals to develop with an inadequate supply of calcium much better then they could otherwise do. This substance is apparently distinct from fat-soluble A, which is essential for growth and which is associated definitely with the prevention of ophthalmia (keratomalacia)." (p. 7.)

In June, 1922, McCollum, Simmonds, Becker and Shipley (56) succeeded in obtaining striking evidence of the existence of a substance in cod liver oil distinct from butter fat which causes the deposition of lime salts in the bones of rats rendered rachitic by the diet. The proof depended on the use of the method developed by Hopkins (57) for the destruction of fat-soluble A in oils already described in connection with Mellanby's experiments, and of the so-called "line test" by which the power of any food substance to cause the deposition of lime salts in the bones could be determined. McCollum, Simmonds, Shipley and the present writer at the very outset of their work had demonstrated that the sudden introduction of cod liver oil into the diet of a rachitic rat is followed by a beautiful deposition of lime salts in a transverse line across the cartilage (74). This phenomenon was utilized for the development of a delicate biological test for lime salt-depositing substances. The four investigators previously mentioned first destroyed fat-soluble A in cod liver oil according to the method of Hopkins. They, then, having proved the destruction of fat-soluble A by demonstrating the ineffectiveness of the cod liver oil for the cure of xeropthalmia, fed it to rats rendered rachitic by means of the diet and discovered that its power to produce deposition of lime salts was retained.

It is difficult to escape the conclusion that a factor or factors exist in cod liver oil distinct from fat-soluble A which cause lime salts to deposit in rachitic bones.

X appears to be closely associated with and related to fat-soluble A. A reason which made Mellanby think the "anti-rachitic dietary factor" identical with fat-soluble A was that the power possessed by various fats to prevent the development of rickets, as demonstrated in his experiments, goes hand in hand with their known content of fat-soluble A, as shown by others. In other words, fats known to contain fat-soluble A appeared also to possess the property of preventing, at least

in some measure, the development of rickets. A second reason which inclined Mellanby to favor the identity of the "anti-rachitic vitamine and fat-soluble A was that the dependence of the animal on fat-soluble A, on the one hand, and on the unknown "anti-rachitic factor," on the other, varies directly with the age. As animals grew older, they tended to become less dependent on both. Of course, the correspondence between the content of fat-soluble A in certain fats and their power to cause lime salts to deposit in the bones of the subjects of rickets does not seem to be so close as Mellanby supposed. McCollum, Simmonds, Becker and Shipley (56) have presented evidence to show that cocoanut oil lacks the power to cure xerophthalmia, but has the power to cause the deposition of lime salts in the rachitic skeleton. Butter fat seems to possess a power to prevent or cure xerophthalmia in excess of its power to prevent or cure rickets, as will be discussed more fully below. In general, however, as far as our limited knowledge extends, it can be said that fats containing fat-soluble A tend to contain also *X*. The greatest argument, however, for thinking that the fat-soluble A and the substance operative in rickets might be the same is to be found in the results of a splendid investigation by Zucker, Johnson, and Barnett (75). These investigators hydrolyzed cod liver oil with sodium hydroxide and separated the fatty acids. The fatty acids were found to be entirely inactive when put to the test on the rachitic rat. The residue, however, exerted a marked curative action. The bases were then removed from the residue and shown to be inactive. The cholesterol fraction was next crystallized from the residue and found inactive. Finally, a fraction was left which gave a curative effect a little stronger than the original oil when diluted with ninety parts of cottonseed oil. This fraction had retained its power to cure xeropthalmia. If Mellanby and the committee of the Medical Research Council which supported him can be accused of "barking up the wrong tree," they at least deserve the great credit for having "barked up a tree" which stood close by the right one. As a matter of fact, Mellanby never stated that the "anti-rachitic vitamine" and fat-soluble A were identical, but that *most probably* they were identical.

The distribution of X in foods. Knowledge concerning the distribution of *X* in foods is limited. *X* is present in large amounts in the oil of the cod and in a considerable amount in the oils of the shark and burbot (a fresh water fish) (56), (76). It is present in small amount, as compared with cod liver oil, in butter fat. Cocoanut oil contains it. Other vegetable oils studied, among which were olive oil, maize oil and linseed oil, appear to be entirely free from it.

Is X to be regarded as a normal constituent of foods? Zucker, Johnson and Barnett (75) state that rickets cannot be ascribed to a "vitamine deficiency" until the curative agent in cod liver oil has been shown to be a normal component of foods. The fish oils, butter fat, and cocoanut oil are foods. It is already necessary to regard *X* as a component of foods. The only uncertainty relates to the extent of its distribution in foods and its concentration in those foods in which it occurs. Probably *X*, like fat-soluble A, occurs abundantly in green leaves. There is urgent need of further investigation of this subject.

The influence of radiant energy[5] *in the prevention and cure of rickets.* As already said, many observers have noted the tendency of rickets to appear in children who lived in the crowded, dark, ill-ventilated rooms of city dwellings. Most of these observers, however, became fixed by the idea that bad influences were there at work, such as "noxious gases," "infection," and failed entirely to consider the alternative of a powerful preventive and curative force in sunlight. A few did perceive the truth. In a remarkable paper on the etiology of rickets (1890), Palm (88) recognized the full importance of sunlight and gave remarkable recommendations for the eradication of the disease by means of sunlight. Raczynski wrote (89) (1912) "that it is the sun which plays the principal rôle in the etiology of rickets," and gave the first proof of the favorable influence of light on mineral metabolism by an experiment on puppies. One puppy was reared in the dark, the other in the sunlight; both were suckled by the mother. At the end of six weeks Raczynski found the calcium oxide and the phosphorus pentoxide in the body of the puppy reared in sunlight to be greatly in excess of that found in the body of the puppy reared in darkness. Raczynski concluded with this statement: "It is possible to assume that the lack of action of sunlight by influencing in so unfavorable a manner the assimilation of calcium oxide in the young organism is one of the causes of rickets. This is in complete accord with clinical experience." The favorable influence of sunlight in the treatment of rickets has been emphasized by Feer (91), and Neve (90).

Almost twenty years ago Buchholz (92) attempted to treat children with rickets by means of light from artificial sources. He used the "gluhlicht"[6] and reported favorable results in sixteen cases. It is

[5] By radiant energy is meant those radiations chiefly, if not entirely, emitted from the ultraviolet portion of the spectrum which exert the remarkable influence on the metabolism in rickets. The term *light* is avoided because some, if not all, these radiations are invisible.

[6] The carbon filament electric light bulb.

possible that the credit for the discovery of the curative action of light in rickets belongs to him, but, since accurate information concerning his work is lacking, it is necessary to assign this great discovery to Huldschinsky.

In June, 1919, Huldschinsky (93) reported that the ultra-violet ray exerted a curative action in rickets. He had treated four children who had advanced rickets with the mercury vapor quartz lamp, and found that at the end of four weeks it was possible to demonstrate with the X-ray deposits of lime salts at the ends of the long bones of the extremities. After two months the healing seemed almost complete.

In September, 1919, Winkler (94) reported the cure of rickets by means of the X-ray. He used a medium soft tube at a focal distance of about 20 cm. The exposure did not exceed ninety seconds and was repeated every other day. The treatment which was directed against the craniotabetic lesions of the head resulted in the gradual disappearance of all manifestations of the disease. Winkler states that he was able to demonstrate the deposition of lime salts at the ends of the radius and tibia by means of X-ray photographs. Hess, Unger and Steiner (95) have been unable to corroborate Winkler's work.

The discovery by Huldschinsky of the curative action of light in human rickets has been corroborated by Putzig, (96), Karger, (97) Huldschinsky himself (98) in numerous additional experiments, Riedel (99), Sachs (100), Erlacher (101), Mengert (102), Hess and Unger (103), Kramer, Casparis and Howland (104) Chick and her collaborators (105), and many others.

Huldschinsky found that the action of light in causing lime salts to deposit in the bones was general, not local. By raying one arm of a rachitic child, lime salts were made to deposit in the other.

Sachs (100) reported that treatment with the ultra-violet ray cured latent tetany and at almost the same time Huldschinsky (106), and a little later Sachs (100) himself, reported cures of manifest tetany by that means.

Huldschinsky (98), made use of sunlight in combination with the mercury vapor quartz lamp in the treatment of two rachitic children; in some cases Riedel (99) relied mainly on treatment with sunlight and used artificial light only when sunlight was unavailable. Hess and Unger (107), however, first demonstrated that sunlight alone possesses the curative action exercised by the light of the mercury vapor quartz lamp in the rickets of human beings. Hess, Unger and Pappenheimer (108) and Powers, Shipley, McCollum, Simmonds and the present writer

(109) investigated the influence of sunlight in the prevention of rickets in the rat and found the prevention to be complete. When the rats were kept out-of-doors in the sunlight, diets, which regularly produced rickets indoors in a medium of roomlight, failed entirely to produce the disease. Later these two groups of investigators (110), (111), (112) further corroborated Huldschinsky's findings by showing that the mercury vapor quartz lamp protects the rat against the development of rickets. Hess, with several collaborators (110), Shipley (140), and Howland and Kramer (76) have reported the protective action of light from other artificial sources known to yield the ultra-violet ray (the carbon arc light, the cadmium spark and the ferric chromium condenser). It is interesting that the tungsten arc which gives a rich line spectrum, extending as far as 210 $\mu\mu$, does not protect rats from the development of rickets.[7]

The radiations which are effective in rickets. The spectrum of the sun is continuous, ranging from the long infra-red to short waves about 290 $\mu\mu$ in length. The mercury vapor quartz lamp has a discontinuous spectrum emitting a number of bright lines, from 595$\mu\mu$ to about 240$\mu\mu$, below which there are a number of weak lines. All investigators, beginning with Huldschinsky, have assumed that the rays of light which exercised the curative action in rickets were the ultra-violet, and such an assumption seemed almost necessary, since window glass filters out of sunlight the ultra-violet radiations, and children behind window glass are not protected from rickets. A multitude of observations and experiments have indicated that the rays of light which produce photochemical reactions lie at the ultra-violet end of the spectrum.

Hess, Pappenheimer and Weinstock (113) have shown that in the case of the rat the rays of light which protect against rickets lie in the ultra-violet zone and are about 300 $\mu\mu$ in length, or shorter. The question exactly which are and which are not the active rays, is complex, since two factors must be considered—the length of the rays and their intensity. It is possible, for example, that rays of light which exert only a feeble effect in the cure of rickets may have great effect if supplied in great intensity as in sunlight. While the general conclusion of Hess, Pappenheimer and Weinstock is not to be doubted, large numbers of experiments must be made with both variables taken into consideration before it will be possible to consider this question as settled.

[7] Results of experiments by Dr. H. Laurens and Dr. G. F. Powers which have never been reported.

ERRATUM

Volume III, No. 1 (January, 1923): Page 127, line 12, instead of "tungsten arc" read "tungsten filament."

When the organism is deprived of the influence of X in the food and of radiant energy defects in the diet become reflected in the blood. This fact is one of the most important of those deduced from experiments on the rat. Diet 761 of McCollum, Simmonds, Shipley and the present writer contained 0.05 per cent calcium and 0.45 phosphorus. According to the analysis of Howland and Kramer (76), the serum of the animals on the diet contained 5.5 mgm. calcium and 8.0 phosphorus per 100 cc. blood. Cod liver oil administered for fourteen days raised the calcium to 8.2 mgm. Diet 618 contained 1.22 calcium and 0.3 phosphorus. The serum of rats on it showed 10.5 mgm. of calcium and 2.4 mgm. of phosphorus per 100 cc. blood. In five days cod liver oil raised the phosphorus to 5.7 mgm. per 100 cc. blood.

In the absence of X and of radiant energy, rickets can be made to develop by altering the composition of the diet in several ways. Undoubtedly the composition of the organic portion of the diet, quite apart from its content in *X*, exerts an important influence in determining the development or non-development of rickets. Unfortunately, the experiments of the New York group of investigators throw little light on this aspect of the question, because they used the same basal ration in all their experiments. McCollum, Simmonds, Shipley and the present writer, on the other hand, used a great variety of diets. Their experiments established the fact that the organic portion of the diet (quite apart from its content in *X*) *may* exert a determining influence. It seems probable that an excessive proportion of carbohydrate in the diet and an insufficient amount of protein or protein of poor quality favors the development of rickets, and, indeed, that any defects in the composition of the organic portion may favor its development if they are of such a nature as to make the diet fail to meet the nutritional needs, and at the same time do not prevent growth of the skeleton. Since no positive information exists to the contrary concerning the influence of the organic constituents of the diet (apart from *X*), one must at present assume that a faulty composition of the diet in respect to fats, carbohydrates and proteins acts chiefly in an indirect manner through its influence on the organism as a whole.

In contrast to the uncertainty which surrounds the question of the influence of the organic components of the diet, no doubt exists that the salt composition of the diet exercises a determining influence for or against the development of rickets. Rickets has been produced:

1. By diminishing the phosphorus content of the diet and administering calcium in the form of the carbonate or lactate or chloride in

disproportionately large quantities (62), (66), (68), (64). The rickets produced in this way is characterized by a low blood phosphorus and a histological condition corresponding to that ordinarily found in the human being.

2. By diminishing the calcium and maintaining the phosphorus content of the diet at a higher level than that of calcium (64), (77). The rickets resulting from diets of this kind has certain distinctive features. The arrangement in the metaphysis of the bone is more orderly than in the low phosphorus form of the disease; evidences of resorptive activity are marked; cells from the fixed tissues with basophilic granules lie scattered about in the immediate vicinity of the trabeculae. No doubt, however, can exist concerning the identity of this form of rickets with the rickets of the human being, and chemical analyses of the blood of the human being are bringing to light proofs of the existence of rickets in which the calcium is low.

3. By adding magnesium carbonate to the diet.[8] McCollum, Simmonds, Shipley and the present writer produced rickets of the greatest severity, by the addition to the diet of magnesium carbonate in quantities varying between 1 and 4 per cent. The rickets corresponded in its general features to the low phosphorus form of the disease. The magnesium carbonate was most effective in diets in which the phosphorus was low. If given in large amount, it had so deleterious an influence on the health of the animal that life was not continued for more than a few weeks.

4. By the addition of strontium carbonate to the diet (78). By the addition of strontium carbonate in amounts equalling 2 per cent, an extreme rickets of the low phosphorus variety was produced. Animals on this diet almost invariably developed paralysis of the hind legs. The condition seemed to be fundamentally different from that which Lehnerdt (79) obtained by feeding strontium. That investigator used diets relatively high in phosphorus. The effect of the strontium was complicated, therefore, by the effect of a high phosphorus.

Experience has shown that not all diets deficient in *X* and having unusual calcium phosphorus balances regularly produce typical rickets. Undoubtedly there are many factors in the diet about which nothing is known, and the question whether these factors are to be found in the organic portion of the diet or in the inorganic portion must remain unanswered pending further investigation. At any rate, in an im-

[8] These experiments have never been published.

portant paper just published, Zucker, Johnson and Barnett (75) report that by making the Sherman-Pappenheimer diet acid through the substitution of calcium chloride in equivalent quantities for the calcium lactate, the rickets-producing power of the diet was removed, and that by adding ammonium chloride in amounts equalling 2 per cent of the total ration its rickets-producing power was lost. A result, the reverse of the ones just recorded, was obtained when a diet which was made up of flour, egg albumin and a suitable salt mixture was used. This diet did not produce rickets. When, however, sodium carbonate was added in an amount equalling 2 per cent, this diet was converted into a rickets-producing one. The experiments of Zucker, Johnson and Barnett indicate that a determining factor for or against the development of rickets is the hydrogen ion concentration of the diet. The work just mentioned must be corroborated. McCollum, Simmonds, Shipley and the present writer succeeded in obtaining rickets of the low calcium variety when the ash of the diet was acid or alkaline. They obtained rickets of the low phosphorus variety when calcium chloride was used instead of calcium carbonate. The experiments of Zucker, Johnson and Barnett, however, are important, not alone from the fact that they indicate another way by which the equilibrium between the calcium and the phosphate ions of the blood may be upset so as to give rise to rickets, but also because they suggest that defects in the salt composition of the diet, other than the disproportion between the calcium and phosphorus, may be significant. When diets characterized by disproportions between the calcium and phosphorus are fed to animals in the absence of *X* and radiant energy, the resulting change in the levels of calcium and phosphorus of the blood is due to a direct action. Undoubtedly, however, the calcium ion or the phosphate ion of the blood can be depressed by indirect action.

In the absence of X and radiant energy, rickets can be prevented by means of the diet. The effect of the organic portion of the diet, quite apart from its content of *X*, on the development or non-development of rickets has already been discussed. Here it is necessary merely to restate the general principle that, if the organic portion of the diet is composed in such a way as best to meet the requirements of the animal and is supplied in sufficient amount, it undoubtedly contributes protection against rickets. Again it is necessary to emphasize the extreme limitations of our knowledge concerning the specific effects of the organic components of the diet.

The fact, however, that rickets can definitely be prevented by the salt composition of the diet is of great importance. For example, the salt mixture 185 devised by McCollum (66) effectually prevents the development of rickets when substituted for a salt mixture which leads to the development of rickets. Sherman and Pappenheimer, it will be remembered, converted their rickets-producing diet into one which did not produce rickets through the simple addition of 0.4 per cent alkaline potassium phosphate, which restored the calcium phosphorus ratio in the diet to one within normal limits. If the work of Zucker, Johnson and Barnett proves to be correct, it is possible to make the diet lose its property to produce rickets by making it acid. Doubtless, as our knowledge of the interrelationships between the diet and the production of rickets increases, it will be found that the disease can be prevented by means of the diet in a variety of ways.

Before leaving the subject, it is advisable to point out the influence of fat-soluble A on the development of rickets. In the diet used by Sherman and Pappenheimer, and in diet 3127 used by McCollum, Simmonds, Shipley and the present writer, the quantity of fat-soluble A present was insufficient to prevent the development of xerophthalmia, and rickets developed. It is possible, therefore, to obtain rickets, even though fat-soluble A in the diet is reduced to a minimum. The rickets never reaches the degree of development under that condition, however, which is possible when fat-soluble A is present in the diet. Apparently when a young, rapidly growing animal is suddenly placed on a diet which is defective in fat-soluble A and is incapable of maintaining growth, growth continues for a time as the result of what may be termed a growth momentum. During this period the salt defect in the diet, if such is present, manifests itself sooner than the defect in fat-soluble A. The new cartilage and bone tissue are formed without lime salts, and a state of rickets is brought about. Pappenheimer and his collaborators and McCollum, Simmonds, Shipley and the present writer found that small quantities of butter added to the diets deficient in fat-soluble A made the rickets more pronounced.

Butter fat is exceedingly poor in its content of X as compared with cod liver oil. Mellanby noted that butter fat was inferior to cod liver oil in its power to prevent rickets, and the only reason that Findlay, Paton and their associates did not fail entirely in the assault on Mellanby's position was the chance choice of butter fat rather than cod liver oil as the substance representing the "anti-rachitic factor." If they had used a potent cod liver oil in sufficient quantity, not one puppy would have become rachitic in any of their experiments.

The proof that butter fat is feeble in its power to cause lime salt deposition in rickets appeared early in the work of the American investigators. At the same meeting, May, 1921, Pappenheimer (80) announced that the addition of butter to the Sherman-Pappenheimer diet in the proportion of 0.2 gram per 100 grams of ration (an amount barely sufficient to prevent xerophthalmia) did not prevent the development of rickets, and the writer reported that butter fat, added to the rachitic diet in quantities equalling 10 per cent of the total ration, was insufficient for that purpose. McCollum (81), (73) also presented a paper which showed from a totally different standpoint the inferiority of butter fat as compared with cod liver oil. When the calcium of the diet was low, butter fat was markedly inferior to cod liver oil in its power to cause growth as well as to induce calcification of bone. Butter fat to 20 per cent of the diet failed to cause satisfactory growth in the presence of a marked calcium deficiency in the diet, whereas cod liver oil was effective in an amount equalling 3 per cent of the diet. In subsequent publications Pappenheimer, McCann and Zucker (65) proved that the addition of butter fat to the amount of 10 per cent of the diet neither prevents nor causes rickets. McCollum, Simmonds, Becker and Shipley (56) showed by means of the "line test," (82) that butter fat must be fed in proportions of 15 to 30 per cent of the diet in order to induce lime salt deposition in the cartilage.

Cows' milk cannot be regarded as an "anti-rachitic" food. The fact that butter fat is feeble in its anti-rachitic action is of practical importance; it means that cows' milk is not a strongly anti-rachitic food, and explains clinical experience which has taught on every side that a diet of cows' milk and rickets are entirely compatible. In this connection the work of Shipley, McCollum, Simmonds and the present writer (72), (73) are of significance. This work showed that cod liver oil possesses in far greater degree than butter fat the power to aid the animal in his struggle against the adverse conditions imposed by diets low in calcium. Evidently butter fat is designed to operate only in the medium in which it is contained.[9] It is possible that the particular combination of salts, protein, carbohydrate and fat which compose cows' milk may be such as actually to favor the development of rickets when introduced into the human organism. The fact cannot be forgotten that the great majority of children who develop rickets do so on diets of cows' milk.

Will cod liver oil invariably cure rickets? In the rickets produced in the rat by diets high or relatively high in calcium and low in phosphorus,

[9] View also expressed by Mellanby.

the low phosphorus form of rickets (62), (63), (64), (66), (68), (83) cod liver oil restores the histological condition in the bone essentially to the normal in from fourteen to twenty-one days. The trabeculae may be fewer than in the bones of the rat on a normal diet but are completely calcified. The provisional zone of cartilage is completely calcified. The bones of the skeleton remain smaller than in rats on satisfactory diets and in comparison with those of the latter are thin.

In the rickets produced by diets in which the calcium is low and the phosphorus higher, the low calcium form of rickets (64) (77), (83), cod liver oil never restores the finer structure of the bone to the normal. The trabeculae do not become completely calcified, and the provisional zone of cartilage is not always completely calcified. The arrangement of the cells in the proliferative zone of cartilage is, however, regular, showing that the orderly growth of the cartilage does not depend on calcium deposition between the columns of cells; the arrangement of the trabeculae in the subchondral portion of the bone is orderly. The trabeculae are greatly increased in number. The reason that cod liver oil does not restore the finer structure of the bone to the normal in the presence of a relative calcium defect, lies in the fact that the calcium depot of the body is in the skeleton, and it becomes necessary for the organism continually to draw upon it. Everywhere in the trabeculae the destruction of old bone and the formation of new, the *umbau*, go on with increased rapidity. Obviously a circulation of calcium is set up through the intermediary of the blood from parts of the bone in which it is not needed to the points where it is most needed, viz., the cartilage and adjacent portion of the shaft. The holes in the trabeculae, many of which contain calcified fragments and free osteoblasts, and the osteoid borders along the trabeculae are merely evidences of the conservation process which the organism resorts to in the face of a calcium deficiency. Cod liver oil cannot ever be thought of as initiating any new process in the bone, but merely as accelerating those which are natural to the organism (84), (85).

Though cod liver oil cannot be said, as judged by histological appearances, to restore the abnormal condition in the bone induced by calcium starvation to the normal one, it does so for all practical purposes, for the gross deformities of the skeleton disappear, and the bones become strong enough to meet the requirements of the animal. Only in rats of the second or third generation on low calcium diets, containing cod liver oil, do fractures regularly develop. In such animals 100

or more green-stick fractures have been counted in the ribs. The bones of the extremities remain exceedingly thin but intact.[10]

When both calcium and phosphorus are low in the diet, and cod liver oil is administered, the histological structure of the bone seems to be determined by the calcium deficiency and is most closely allied to that already described for the low calcium form of rickets.

When rickets is produced by the addition of strontium carbonate to the diet (78), a form of rickets which probably never occurs in the human being, cod liver oil exerts little influence. In the rickets induced by the administration of magnesium carbonate, also, cod liver oil exerts a limited influence. It is possible that the rickets from excessive quantities of magnesium in the food may occur in animals (86).

When Findlay (87) states that cod liver oil does not cure the rickets of the human being, one infers either that the cod liver oil used was not potent or was not actually taken. One can be certain that in the human being with rickets, with the possible exception of the premature infant, cod liver oil never fails to exert a curative action without change in the living conditions or diet.

The effects and mode of operation of cod liver oil and radiant energy. What cod liver oil and radiant energy actually accomplish is beautifully illustrated in experiments performed by Powers and Guy.[11] These investigators placed large series of rats, abundantly controlled and living in a medium of roomlight, on a basal ration which was defective in *X*, calcium and phosphorus. In the diets fed two sets of animals the calcium was made high and the phosphorus kept low. In the diets fed two other sets the calcium was kept low and the phosphorus was made high. When these diets had been administered long enough for the disproportions between the calcium and phosphorus to become reflected in the blood, as was determined by chemical examination, cod liver oil was administered. In those animals in which the calcium of the serum was low, cod liver oil caused the calcium to rise to the normal level, restoring a normal equilibrium. In those animals in which the inorganic phosphorus in the serum was low, cod liver oil caused the inorganic phosphorus to rise to a normal level, establishing a normal equilibrium. Obviously cod liver oil acted as a regulator of the mineral metabolism, at least in so far as the calcium and inorganic

[10] These experiments by McCollum, Simmonds, Shipley and the present writer have never been reported.

[11] These experiments have not yet been reported.

phosphorus are concerned, and one may infer that a function of cod liver oil and also of radiant energy is to maintain the normal salt equilibrium of the body in the presence of salt combinations of different complexities which are continually being absorbed from the food.

Though we may only conjecture concerning the mode of operation of cod liver oil and radiant energy in rickets, certain observations concerning their effects are suggestive. Animals on rickets-producing diets, which are given cod liver oil and kept in the sunlight, grow better, are more active, more nearly normal, and live longer than their control animals. Obviously the beneficial effect of the energy derived from the sun and of cod liver oil is not limited to the rachitic process. Also, the favorable effect of cod liver oil and radiant energy on rats receiving diets which give rise to rickets is maintained as long as either is supplied.[12] The examinations by Howland and Kramer (76) of the blood of the animals used in the experiments of McCollum, Shipley, Simmonds and the present writer have shown that cod liver oil maintains the level of the calcium in the blood near to the normal in rats kept for four generations on diets so deficient in calcium as to render the animals in a perpetual state of calcium starvation. Neither cod liver oil nor radiant energy supplies the required calcium or the phosphorus. It is an extraordinary fact, however, that either one can enable the rat, kept in a state of calcium or of phosphorus starvation, to obtain so good a utilization of the minimal quantities of calcium or of phosphorus supplied in the food as to maintain the level of the calcium or of the phosphorus in the body fluids almost at the normal. Without supplying either phosphorus or calcium radiant energy or *X* causes the organism to operate as if a requisite or almost requisite quantity of phosphorus or calcium were supplied. They do so by supplying something which makes the metabolism more efficient, i.e., they cause the organism to operate with increased economy. Neither the histological studies of the bones nor the chemical examinations of the blood indicate that either cod liver oil or light bring new processes into operation, but rather permit the organism to have full use of processes which were natural to it all the time, but were not effective.

[12] The question arose: Did not cod liver oil or radiant energy exert their favorable influence in rickets by making available to the organism depots of phosphate or calcium which previously had been inaccessible? On that supposition one would expect the rickets to recur when the depot of phosphate or calcium finally became exhausted, even though the administration of the cod liver oil was continued.

Radiant energy is a powerful oxidizing and reducing agent in the case of the simpler chemical compounds, and probably exerts an influence of some such general nature in man and animals. It communicates to the organism energy in some form which is apparently essential for normal metabolic activity, in particular in the growing organism. It is probable that cod liver oil operates in some such general way.

Apart from rickets cod liver oil and sunlight cannot be considered to have an equivalent action. Powers, Simmonds and the present writer (114) have shown that radiations from the mercury vapor quartz lamp have almost no influence in the prevention of xerophthalmia in the rat, whereas cod liver oil has a powerful preventive action. They found that sunlight and fresh air, on the other hand, delayed the development of xerophthalmia in some animals and in others prevented it over periods of four or five months. Obviously, however, sunlight and fresh air had kept the xerophthalmia only just submerged, since it quickly made its appearance when the animals were removed from sunlight to ordinary roomlight. Cod liver oil prevents xerophthalmia more easily than it prevents rickets; sunlight, however, prevents the development of xerophthalmia only with difficulty but readily prevents the development of rickets. The investigation just mentioned speaks rather in favor of the existence of at least two factors in cod liver oil, the one exercising a special influence in the prevention and cure of xerophthalmia and the underlying disturbance in metabolism, the other exercising a special influence in the maintenance of the normal salt equilibrium in the blood. It also shows that cod liver oil furnishes a factor the equivalent of which sunlight does not supply, or supplies in small amount. Abundant evidence exists that radiant energy produces effects which cod liver oil cannot bring about. So far as the influence on the mineral metabolism in rickets is concerned, however, their functions seem to overlap. It is difficult to conceive that *X* so abundantly contained in cod liver oil, and radiant energy which exercise this extraordinary influence on the metabolism of the organism, should be entirely unrelated. Doubtless the future will show that some intimate connection exists between them.

Effects of starvation. The attention of McCollum, Simmonds, Shipley and the present writer (115) was early called to the fact that animals which grew poorly and presumably ate poorly did not show rickets. The thought, therefore, suggested itself that starvation might excite healing. Accordingly, rats that had been rendered rachitic by means of diets low in phosphorus and fairly high in calcium carbonate

were starved for five day periods. When they were killed, beautiful deposits of lime salts comparable to those found in rats giving a positive "line test" to cod liver oil stretched across the border of the cartilage at the rachitic metaphysis. Observations which have the same significance were made by Pappenheimer and his collaborators.

The starving animal no longer carries the load of the unbalanced diet. His tissues, breaking down, liberate substances required to restore the normal equilibrium, including perhaps *X* itself. Many studies of the metabolism in starvation have shown that the body in that state uses its resources with the utmost economy. It is not strange, therefore, that under the influence of starvation the normal equilibrium reforms. The phenomenon has its analogue in the favorable influence of starvation on the metabolism in diabetes and other diseases.

The relation of growth to the development of rickets. For the development of manifest rickets and osteomalacia growth is essential. Both are diseases intimately concerned with the phenomenon of growth. The peculiar turn which the metabolism takes in rickets is probably dictated by the requirements of growth, and in this way is explained the frequency of rickets during the periods of rapid growth.

So many writers have pointed out the fact that malnourished infants do not become rachitic, that one need only mention the fact here. Sometimes, however, the subjects of extreme rickets are emaciated. Jundell (25) has been so impressed by the apparent relation between rickets and growth that he has revived the idea originally advanced by Glisson which ascribes rickets to over-eating (over-nutrition).

Is the rickets produced experimentally in animals the same disease as rickets in the human being? Findlay (60) questions whether the rickets induced in animals is the same disease as the rickets of the human being. In reply these facts may be cited: The lesions in the skeleton, both gross and minute, are identical; the pathological conditions found to exist in the blood are identical; the rickets produced experimentally in animals may or may not be accompanied by the symptoms of tetany, exactly as rickets in the human being may or may not be accompanied by tetany; the same remedial measures are effective in both. Not the slightest doubt can exist that the rickets produced experimentally in animals and the rickets occurring in human beings is the same disease.

THE CHEMICAL CHANGES OCCURRING IN RICKETS: *The changes in the blood occurring in rickets.* A great step forward was accomplished when Iversen and Lenstrup (116), and Howland and Kramer (117), quite independently, reported that in ordinary rickets (rickets uncomplicated

with tetany), the inorganic phosphorus in the blood is low. Howland and Kramer found that in non-rachitic infants the concentration of calcium is from 10 to 11 mgm. per 100 cc. of serum and of inorganic phosphorus about 5 mgm. During the period of active rickets the calcium concentration of the serum may be normal or slightly reduced, a result in agreement with that previously reported by Howland and Marriott (118). The inorganic phosphorus of the serum, however, is regularly reduced, in some cases to an extreme degree, for example to 1.0 mgm. With the exception of that form of tetany which is induced in infants through the administration of the sodium carbonates or by over-ventilation of the lungs, infantile tetany must be regarded as a symptom-complex occurring in rickets. It is, therefore, necessary to inquire into the conditions prevailing in the blood in tetany. Howland and Marriott (118) some time ago found that the calcium of the blood is considerably reduced in infantile tetany, exactly as in the tetany of animals caused by extirpation of the parathyroid glands. During active symptoms the calcium of the serum may fall to 3.5 mgm. per 100 cc. of blood. The inorganic phosphorus is, however, usually "normal" varying from 3.6 to 5.8 mgm. per 100 cc. The concentration of sodium and magnesium was found to be essentially "normal," that of potassium slightly increased. When calcium is administered in tetany in the form of calcium chloride, the calcium in the blood serum rises. In the human being and also in the rat the rise of the calcium, however, is frequently associated with a fall in the inorganic phosphorus. These findings in the blood in ordinary rickets and in rickets accompanied by tetany have received abundant confirmation (Findlay, Paton and Sharpe (1), Hess (119), György (120), von Meysenbug (121) and others).

Iversen and Lenstrup reported that the inorganic phosphorus of the blood was increased after the administration of cod liver oil. Howland and Kramer also reported its regular increase after the administration of cod liver oil to the normal level and "often somewhat above this." Apparently the rise in the inorganic phosphorus of the blood began to be apparent after fourteen days of treatment and did not become complete until four to six weeks had elapsed. Radiant energy exerts an influence on the level of the inorganic phosphorus of the blood in rickets which is identical with that of cod liver oil. Hess and Gutman, (107) Hess, Unger and Pappenheimer (110) and Howland and Kramer (76) have reported that radiant energy, whether derived from the sun or artificial sources rich in ultra-violet radiations, cause the low inorganic phosphorus of the serum in rickets to rise.

In this connection the reports of the chemical examinations of the blood made by Howland and Kramer (76) on the rats used in the experiments of McCollum, Simmonds, Shipley, and the present writer are of the greatest interest. The findings in the blood corresponded in a wonderful manner with the histological findings and have both supplied new information and given splendid support to the conclusions drawn from the latter. The chemical examinations of the blood showed: *a*, that the concentration of the inorganic phosphorus and calcium of the serum cannot be made to exceed the concentrations regarded as normal; *b*, that when the concentration of either calcium or inorganic phosphorus in the serum is low, it may be increased by increasing the amount of the respective element in the diet; *c*, that when the inorganic phosphate of the serum is low it may be increased by starvation, the addition of phosphorus to the diet in organic or inorganic form or of various fats (the fish oils and butter fat), and by exposure to radiations of the requisite quality. The examinations of the blood also indicated that cod liver oil raises the calcium of the serum in the rat when the latter is low.

The metabolism in rickets. Knowledge concerning the metabolism of rickets is so confused that the wisdom of discussing it beyond the point of obtaining a general orientation seems doubtful. Reviews of the subject will be found by Orgler (122), Lehnert (123), and Paton, Sharpe and Findlay (1). Two splendid studies to which the reader is especially referred are those by Telfer (124) and Hamilton (125). The one by Hamilton was made on the premature rachitic infant.

The disturbances in the salt equilibrium in the blood which characterize rickets have already been described. The total ash of the bones is greatly reduced. For example, Telfer found the mineral matter of the dried bones of the leg of a rachitic dog to be 17.7 per cent, as compared with 44.9 per cent in the normal animal. Brubacher (126) gives similar figures for the rachitic and normal child. Another important fact is that in the rachitic subject the ratio of calcium to phosphorus in the bone does not differ materially from that in the bone of normal individuals. Both Telfer and Hamilton have shown that in rickets the amounts of calcium and phosphorus retained are less than in healthy persons. This point in the metabolism of rickets has been definitely established. Schabad (127) has reported negative calcium and phosphorus balances in rickets and it is probable that in rickets negative balances may occur. Another interesting point brought out by Telfer is that the proportion of phosphorus retained is less than that of cal-

cium. A fact which has especial importance in regard to the metabolism of the premature infant with rickets and all infants on diets of breast milk was brought out by Hamilton. He showed that the normal infant excretes about 0.200 gram of calcium oxide daily, regardless of the intake or weight. For an adequate calcium retention to occur, therefore, it is necessary that considerably more than that amount be supplied in the food. There is no good evidence that fat, protein, or carbohydrate, considered as such, influence calcium metabolism. Schabad (128), Schloss (129) and Orgler (122) have reported that cod liver oil increases calcium retention; Freund (130), Orgler and others have performed experiments tending to show that milk fat decreases calcium retention. Telfer failed to find that either butter fat or cod liver oil influenced calcium retention.

The metabolism of rickets must be studied anew. In most investigations the periods of study have been too short, and the condition of the child during the experimental period has not been sufficiently taken into account. The fact that *X* in cod liver oil and other substances does not seem to produce its effect until a period of days has elapsed has not been taken into account. In view of the remarkable influence of radiant energy on the mineral metabolism, fresh experiments on normal infants ought to be carried out in order to determine normal standards.

There are several explanations for the diminution in the retention of calcium and phosphorus in rickets. One is that the calcium and the phosphorus are not absorbed from the intestinal tract. On this supposition it is likely that either the digestive functions are disturbed in some peculiar way, or else the combination of food substances is such that insoluble products incapable of absorption are formed, for example, tri-calcium phosphate or calcium soaps. Another possibility is that the calcium and phosphorus are absorbed, but, on account of abnormal conditions in the internal metabolism, are not allowed to accumulate in the blood; they are either driven out of the body or are used to carry out some other substance. Under this latter supposition it is necessary to suppose the primary disturbance to be in the internal metabolism. In either case it seems altogether probable that the deposition of lime salts in the bones is determined chiefly, if not entirely, by the products of the concentration of the calcium and phosphate ions in the circulating fluid as Howland and Kramer (131) have indicated. When the concentrations of calcium and phosphate in the blood increase sufficiently, the deposition of lime salts apparently

occurs. Telfer and Zucker seem to incline to the view first mentioned. If, however, the calcium and phosphorus are not absorbed from the digestive tract, is it not because the internal metabolism does not demand their absorption? Even if they are not absorbed, it does not follow that the disturbance is not in the internal metabolism which may have failed to create the necessary demand. It seems easier to explain the action of radiant energy and also of cod liver oil in rickets on the ground that the metabolism of the whole body is altered, rather than on the basis of a peculiar composition of the food or a local disturbance in the digestive system. We should beware, however, of drawing positive conclusions in a subject about which so little is known. Moreover we ought to remember that such a disturbance in metabolism as the one occurring in rickets probably does not always come about in the same way.

The social and living conditions of rachitic children. Rickets occurs most commonly among the children of the poor, who eat poor food and spend much of their time indoors in poorly-lighted, badly-ventilated, dark rooms. These are facts emphasized by Kassowitz and many others. It is important to remember, however, that rickets not infrequently makes its appearance in families of means living under conditions of hygiene and diet which have been regarded as above criticism. Instead of generalizing further, it seems advisable to give brief résumés of the two best studies on this subject, the one by Ferguson (24) in Glasgow, the other by Hutchinson and Shah (132) in India.

Ferguson investigated two hundred families in which marked rickets existed, one hundred and fifty families in which there was mild rickets, and one hundred families in which the rickets appeared to have been cured. She compared the conditions found with those present in two hundred families in which the children were free from rickets. The chief points brought out were: Inadequate air and exercise appeared to be potent factors in determining the onset of rickets. In the rachitic families the social conditions were somewhat the worse. The average number of persons per room was almost one greater; the cubic space per person was 32 per cent less; the houses were less cleanly, and the children were taken out of doors in the fresh air considerably less. ("Over 40 per cent of the rachitic children had not been taken out, while only 4 per cent of the non-rachitic children had been confined indoors.") Ferguson's study of the dietaries in the rachitic and non-rachitic families was not altogether satisfactory. In general, the diets in the non-rachitic families had about the same energy value as

those in the rachitic families. In the non-rachitic families the average amount of fat in the diet was 10.7 per cent higher; the consumption of milk was somewhat greater; that of fish and eggs double; and of margarin or butter slightly greater than in the rachitic families. Though Ferguson minimized the differences in the diets in the two groups, the diet of the non-rachitic families was the better. The study by Ferguson was an extension and corroboration of a similar study made by Findlay several years previous.

In their most remarkable investigation of the effect of hygiene versus diet upon the development of rickets, Hutchinson and Shah made direct comparisons of diets and living conditions among the Mahomedans and Hindus of the laboring classes with those prevailing among the higher caste Mahomedans and Hindus, among whom the practice of the "purdah" was usual. The women, as well as the men of the laboring classes, worked in the fields, and while at work left their young infants at some nearby point in the open air. The houses of this class were "one roomed, ill built huts with a hole in the roof for a chimney." Among the higher caste Hindus and Mahomedans the infant remained, as a rule, for the first six months of his life with his mother in a semi-dark, ill ventilated room in the interior of the house, and ordinarily remained indoors until able to walk and look after himself. The women usually married at the age of twelve, entered "purdah" and from that time on left the house only for short periods on special occasions. The houses were built in such a way as to admit little light and air. The dietaries of the higher caste Hindus and Mahomedans were considerably better than the dietaries of the laboring class. They contained more animal fat, butter, eggs and milk. Fresh vegetables figured little in the dietaries of either. The point of the investigation was that rickets occurred scarcely at all among the infants of the laborers, whereas it was exceedingly common among the children of the upper caste families. Moreover, among the women of the upper castes late rickets was frequently encountered.

In reality the investigations of Hutchinson and Shah showed merely that sunlight prevented the development of rickets. The diets of both castes were deficient in the factor *X* and poorly constituted. The children and women of the laboring class were protected by sunlight. Those of the higher castes lacking the influence of sunlight developed the disease.

Diets of rachitic children. Until quite recently it was taught that diets in which the carbohydrate was in excess, the fat deficient, and

perhaps also the protein, produced rickets. This view arose from the observations of physicians that rickets was particularly apt to appear in children receiving condensed milk and proprietary foods. At the same time the theory found support in the reports of experiments by Bland-Sutton at the Zoölogical Garden. The so-called experiments of Bland-Sutton have had an influence which apparently they have not merited. Cheadle and Poynton (133) wrote concerning them in 1908: "The history of these lion cubs is very significant; with the exception of a single litter, suckled by the dam ten years before, the cubs brought up on horse-flesh in this way invariably died,—the cause of death being, as invariably, extreme rickets. More than twenty litters had been lost in this way. The feeding of the last litter of lion cubs was begun in the usual fashion. The dam had very little milk, and at the end of two weeks the cubs were weaned entirely and were then put on horse-flesh as usual. They quickly became rickety, and the muscular weakness, as well as bony deformity, were extreme. The malady advanced rapidly and one cub died. Then, by the advice of Mr. Bland-Sutton, milk, pounded bones, and cod liver oil were added to the raw meat, which was continued exactly as before; they were kept in the same dens with the same amount of warmth and light and air, and, with the single exception of the addition to the diet, no change of any kind was made in the regimen. The change in nutrition which followed was immediate and remarkable; in three months all signs of rickets had disappeared, and the animals grew up strong and healthy—a unique event in the history of the society. The experiment seems a crucial one, and decisive as to the part played by fat and bone salts, with some casein and lactose, in the production and cure of rickets" (pp. 85 and 86).

Findlay (87) wrote concerning these experiments in 1921: "Bland-Sutton has kindly supplied me with details of his experiences with rickets in young carnivora in the London Zoölogical Gardens, experiences which have been extensively quoted in the medical literature, but which were never published in detail by him. His attention has been drawn to the difficulty of rearing young carnivora in confinement. They 'were often born with cleft palate, and those that survived soon developed rickets.'" To remedy these things, he advised that the adult lions should be given goat flesh and bones twice weekly instead of being fed entirely on horse-flesh (the bones of horses being very hard), and that the young be given cod liver oil to lick. As a result of these changes in diet, some of the lion whelps were born without cleft palate,

and others, which grew up to adult life, presented remarkable rickety changes in their skulls. Such experiments do not justify the conclusions regarding the influence of fat in rickets that have been based on them by his disciples, and I know that within recent years Bland-Sutton has held the opinion that a gastro-intestinal disturbance, probably of a microbic nature, is the initial cause of the disease" (pp. 152 and 153).

Few systematic investigations of the diets of rachitic children have been made. As just mentioned, the investigations of Ferguson indicated that the diets in "non-rachitic" families were superior to those in the "rachitic" families, and those of Hutchinson and Shah showed that the diets in the "rachitic" families were better than in the "non-rachitic."

In January, 1920, Hess and Unger (134) put to the test upon the infant the diet which Mellanby had used with so much success for the production of rickets in puppies. They gave to five infants between four and nine months of age a diet composed of 180 grams krystalak, the fat content of which was less than 0.2 per cent, 30 grams cane sugar, 10 cc. orange juice, 30 cc. autolyzed yeast and 30 cc. cottonseed oil. The infants did not develop symptoms of rickets during observation periods of five to ten months. The experiments were undertaken in order to determine the influence of a deficiency in the diet of fat-soluble A on the development of rickets in the human being. From that standpoint they were not of great value. Fat-soluble A was still present in the food, and clinical observation alone did not make the freedom from rickets certain. It is interesting, however, to learn that this diet seemed to have a greater protective value than diets containing considerable amounts of butter fat, perhaps because of the very deficiency in fat-soluble A.

Chick, Dalyell, Hume, Mackay and Smith (105) have reported concerning the effects of two diets used as standards in Pirquet's clinic, Vienna. The first diet contained about two-thirds to one-half of its total energy in the form of sugar. It was, therefore, high in carbohydrate (65 per cent), relatively low in milk and, hence, milk fat (24) per cent, and in fat-soluble A, especially as the milk used was from stall-fed cows and found to be low in vitamine A by actual test; it was also low in protein (11 per cent). The second diet consisted of full cream dried milk diluted so as to yield 13 per cent solids. It contained protein (20 per cent), fat (45 per cent) and carbohydrate (35 per cent). Additions of cereals were made for older children, and cod liver oil was given to the younger infants. The addition of the

cod liver oil to the second diet was most unfortunate because it destroyed completely the value of any comparative data. It was found, as was to be expected, that the second diet (plus cod liver oil) protected against the development of rickets. The first diet proved curative when cod liver oil was added to it. The most important development from the study was the discovery that children on the first diet (when cod liver oil was not added) developed rickets during the winter and spring in spite of excellent hospital conditions, but did not develop it during the summer.

Several years ago McClure, and subsequently Powers,[13] analyzed the dietaries of infants and young children with rickets who entered Johns Hopkins Hospital. They found that infants and young children developed rickets on all foods ordinarily given. Negro children, who had not shown marked evidences of the disease while being breast fed, developed it in a severe form between the ages of ten months and two years, when they ate table food. The diets were composed of bread, syrup, candy, cake, potato, and little milk. It was fairly common to find infants on diets of sweetened condensed milk who had developed rickets, and others who did not show a trace of the disease. Several children with extreme rachitic involvement of the thorax and multiple fractures had received only diluted cows' milk, and in Dunham, Willis and Guy's[14] series of cases of very early rickets, eight of ten artificially fed babies were receiving such diets. In one extraordinarily severe case of rickets in a negro child the diet consisted mainly of toast and tea. It was common to see rickets in children whose diets of cows' milk, sugar, or starch mixtures had been prolonged into the second year. Hess (135), (136) thinks children are particularly liable to develop rickets on diets of protein milk.

One may state positively that rickets develops on all milk mixtures and on almost all foods or combinations of foods ordinarily given to children. In general, however, the diets of rachitic children, particularly of older children, are open to criticism. Either there is an excessive amount of carbohydrate or an insufficient amount of protein or of fat, or there is insufficient carbohydrate, as in simple dilutions of whole milk, or the protein is of poor quality, or the food value of the diet is below the requirements, or the diet is poor in calcium or in its total salts, or is unsuitable for the digestion or for the age, as when mixtures of milk and carbohydrate are continued into the second year.

[13] These investigations were never reported.

[14] These investigations will soon be reported.

Analysis of the diets of rachitic children, however, does not indicate that salt defects, such as seem to be required for the production of rickets in the rat, are present. In occasional instances the total salts may be low (the protein in that case is deficient also), or the calcium may be low, as in the diets of the negro children previously mentioned. The majority of the diets, however, on which children develop rickets, are based on cows' milk, and the salt composition is that of cows' milk. On the other hand, children may receive diets open to the greatest criticism from several standpoints and yet not develop rickets. Children on diets of carbohydrate alone do not develop rickets. The poorest diets will not cause rickets if they are supplemented with cod liver oil, or if the child is protected by sunlight.

One fact of great importance may be mentioned here. The older the subject of rickets, the greater the probability of marked defects in the diet. In old rachitic children the diet is almost invariably found to be defective.

Rickets occurs in breast-fed children with much greater frequency than generally supposed. A large percentage of negro babies who are breast-fed show signs of rickets, and many breast-fed white children of cities are affected. Rickets, however, does not usually develop in white babies, if breast-fed, and rarely manifests itself in breast-fed babies in severe forms. Findlay and Ferguson found rickets as common in children who had been breast-fed as in those who had never been breast-fed, an observation showing at least that breast feeding confers no lasting protection. Rickets in the breast-fed child is often accompanied by tetany.

A study was made by Hess and Unger (137) of the diets of the negro mother in a negro district of New York City where rickets was exceedingly prevalent among breast-fed children. The negroes were for the most part immigrants from the West Indies where they had lived an out-of-door life and consumed a diet consisting mainly of vegetables, eaten fresh, and fruits. In New York City they lived chiefly on meat or fish, rice and potato, tea, coffee, and cereal. Milk was taken in small quantity and fruit rarely. The vegetables which were taken in small amount were usually canned. In New York City the women lived indoor lives. The striking points brought out in the study were that the mothers had exchanged an out-of-door life in the tropics for an indoor life in the temperate zone, and a dietary in which green vegetables and green leaves figured largely for one in which those articles of food were almost entirely lacking.

In by far the most accurate study thus far made of the dietaries of the nursing mothers of rachitic children, Dunham, Willis and Guy[15] have found that the diets both during pregnancy and lactation are almost always poor and open to a variety of criticism. In the majority of instances the caloric value of the diet was under the requirement for mother plus child, and fresh vegetables and fruit were almost entirely lacking. In five of eleven instances the diet was low in calcium and in two instances in phosphorus. In the one instance in which the amount of green vegetables in the dietary was abundant, the total value was only 1125 calories, and the total phosphorus was under the minimum. It should be added that almost all the mothers of the rachitic infants led indoor lives.

In this connection the observation made by Siegert (30) that the breast-fed infants of rachitic mothers usually have rickets may be significant. Though the conditions of hygiene and diet which caused rickets in the infant do not produce the disease in the mother, they may be conceived of as affecting her to the extent of rendering her milk deficient in its protective qualities. Certainly the studies of Hess and Unger and of Dunham, Willis and Guy show that women receiving diets deficient in fresh vegetables and other foods containing fat-soluble A and probably *X* and living away from sunlight, do not yield breast milk which protects their offspring from the development of rickets. De Wesselow (138) and v. Meysenbug (121) have shown that the milk of the mothers of rachitic infants is not lacking in either calcium or phosphorus.

Discussion. As the result of clinical observation and investigation it has become clear that two factors exist, the one in radiant energy, the other in an unknown form in certain foods, either of which is capable of preventing rickets from developing or from continuing, if already established. It has become equally clear, also, that only when the organism is deprived of the influences of radiant energy and of *X*, can rickets develop. Recent advances in knowledge have failed entirely to disclose the nature of vitamines, or their mode of operation in the organism or the mode of operation of the ultra-violet radiations of the environment. They have revealed, however, certain of the effects upon the organism of radiant energy and of *X*; both exercise a stimulatory or a regulatory influence on the metabolism of the body, in particular of the calcium and phosphorus. One at least of their functions appears to be the protection of the organism from the dangers

[15] This study will soon be reported.

attendant on the absorption and entrance into the blood of substances which might disturb its salt balance. Moreover, increased knowledge has indicated that the rôle played by radiant energy and *X* in the maintenance of the normal salt metabolism is of the utmost importance, and that the organism is dependent on the energy of the sun's rays or of their equivalent in the food to an extent little appreciated. The facts enumerated indicate beyond a doubt that rickets is a deficiency disease. Moreover, they settle for all time the differences of opinion between the English and the Scottish schools concerning the cause of rickets. Rickets is a dietetic disease, since, for its development the diet must be insufficiently supplied with the factor *X*. At the same time it is a disease of defective hygiene, since for its development there must be an insufficient supply in the environment of the radiant energy which exerts the protective action. The facts just mentioned also explain the disagreement among the students of rickets, the world over, concerning the causation of the disease. Obviously two factors have been concerned, but all investigators have proceeded on the assumption that only one factor was concerned, and have perceived so clearly the existence of the one that they have denied the possibility of the existence of the other.

As stated, radiant energy and *X* in the food exert only a protective or curative and regulatory influence. The question naturally arises, therefore, are there factors in the diet or elsewhere which actually can *bring about* the development of rickets? Experiments have proved that in the rat the food, quite apart from *X*, can precipitate the development of rickets (in the absence of protective radiations), or stop its development. In the case of the rat, therefore, no doubt exists that the food can exert a determining influence either for the development of rickets or against its development. Can the diet, then, exert a similar determining influence in the human being? Obviously absolute knowledge is lacking. Not the slightest doubt, however, can be entertained that diets such as do actually produce rickets in the rat would produce rickets in the human being under the same conditions; also, that diets such as prevent the development of rickets in the rat would prevent the development of rickets in the human being. The evidence at our disposal indicates that the human being is more susceptible to the development of rickets than the rat.

Since, then, it seems certain that the diet (apart from its content of *X*) *can* exert a determining influence for or against the development of rickets in the human being, it is necessary next to inquire whether

the diet in the human being *does* exert this determining influence. Do the studies of the diets of children with rickets reveal defects which appear to be capable of precipitating the disease? The studies already referred to indicate that such abnormalities in the salt composition of the diet as seems necessary for the production of rickets in the rat are not ordinarily present in the diets of rachitic children. In some cases of rickets, in particular when older children or adults are affected, defects in the salt composition of the diet akin to those necessary for the production of rickets in the rat may be found. In a great majority of instances, however, variations in the calcium and phosphorus and in the reaction of the diets fall within limits which according to present standards must be regarded as normal. The point which stands out with great clearness in the study of the diets of children suffering from rickets is that no single fault (apart from a possible insufficiency in *X*) can be found which is common to all. The defects appear to be of various kinds and of such nature as rather to predispose to the development of rickets than actually to cause its development. It is true also that the diets on which some children develop rickets seem to be well constituted and do not cause rickets in others.

In this connection a word of warning is required. Knowledge concerning the relationship of different food substances to the organism is in its infancy. Defects in the diet of such nature as actually to be productive of rickets may be present in the diets of all rachitic children. In our present ignorance, however, one cannot recognize them.

Since the analysis of the diets of children developing rickets fails to reveal a constant defect or defects which appear to be capable of *bringing on* the disease, must it not be thought that the human organism is so dependent on the regulatory influence of radiant energy or of *X*, that rickets can develop even when the diet is well constituted,—in other words, that the human organism is extraordinarily susceptible to rickets? No single defect (aside from a possible insufficiency of *X*) is common to the diets of all rachitic children. Some infants develop rickets on foods which do not produce rickets in others. Infants may develop rickets even when a diet is composed of breast milk. Premature infants usually develop rickets, even when the food is breast milk. These facts make it necessary to conclude that the human organism is peculiarly dependent on the presence of radiant energy or its equivalent in the food, and that rickets may develop when the organism is deprived of them, even though the diet be what is commonly considered well constituted.

If the view just stated is true, then rickets is purely a deficiency disease. Is such a conception untenable? Of course, one would not expect to find the relationship between rickets and the diet so obvious as between scurvy and the diet, since in the case of rickets the deficiency in *X* can be compensated for by radiant energy in the environment. Yet if rickets and scurvy are compared, numerous points of similarity are found. In both diseases the incidence among infants is much greater than among adults. Under unusual circumstances great groups of adults have become the victims of scurvy, and, during the great war, numbers of adults in Austria and Germany became victims of osteomalacia. One reason that rickets occurs less commonly in adults than in children is that the adult has a more varied diet than has the child. The chief reason, however, lies in the fact that the adult resists far more those influences producing rickets than does the child. All who have attempted the experimental production of rickets in animals are agreed that old animals are more resistant than young animals to the development of rickets, and that rickets tends to undergo spontaneous cure with increasing age. As already pointed out, the younger the organism, the greater the dependence on radiant energy in the environment or on the factor *X* in the food, and the more intimate the relationship between these factors and the phenomenon of growth. Growth, however, never entirely ceases. The formation of new tissue and the destruction of old goes on continuously, even in extreme old age. Rickets differs from the other deficiency diseases, so far as present knowledge extends, in that the deficiency must be two fold.

There are two great arguments in favor of the conception that rickets is a deficiency disease. One argument lies in the following fact: The striking features in the occurrence and associations of rickets can be explained by the occurrence of radiant energy in the environment or of *X* in the food. The negro and Italian children in American cities are especially susceptible to rickets because the pigment of their skin partially insulates them, so to speak, from the sun's rays, and makes them derive less benefit from that source than do fair skinned children. The diets of the negro and Italian child in our American cities are notoriously lacking in those foods which carry fat-soluble A and presumably *X*. The negro child in Africa does not develop rickets because he lives out of doors and is nursed by a mother who eats food presumably rich in *X* (green leaves). Chinese and Japanese children do not develop rickets because they live out of doors and are breast-fed by mothers whose diets contain foods rich in fat-soluble A and presumably

in *X*. The children of the Esquimaux do not develop rickets, though they live in dark huts and for one-half of the year are in the Arctic twilight, because they are breast-fed by mothers who eat large quantities of fats abounding in *X* and are weaned to diets containing *X*. The seasonal variation of rickets is to be explained on the ground of the seasonal variation in the richness of the solar spectrum in ultraviolet radiations, as pointed out by Hess, but, also, by the seasonal variations in foods which contain *X*. The incidence of rickets varies with the deficiency in radiant energy and *X*, and not with any other factors which our present knowledge permits us to pick out. The association of rickets with scurvy is also in accord with the theory that rickets is a deficiency disease. It will probably be shown that a whole group of foods which are rich in the antiscorbutic vitamine are also rich in *X*.

The other argument which amounts to proof is as follows:—The addition of *X* to the diet or of radiant energy to the environment invariably cures rickets, if established, or prevents its development without change of the diet or conditions of life.

The whole subject of the etiology of rickets is extraordinarily complex, and the clear presentation of the facts, not to mention the correct interpretation, is most difficult. At the risk of falling into pitfalls, however, it seems wise to attempt to draw a parallelism between rickets and diabetes,[16] which will make clear the views of the present writer. Diabetes is a deficiency disease in the sense that an internal secretion essential for the maintenance of normal metabolism is deficient. Rickets is a deficiency disease in the sense that radiant energy and a factor *X* are deficient. Diabetes is a disturbance in the carbohydrate metabolism. Rickets, as we know it, is a disturbance in the metabolism involving in particular the calcium and phosphorus. It is possible to influence diabetes by means of the diet in two ways: directly, by feeding diets rich in carbohydrate, substances which the organism finds it most difficult to metabolize; and indirectly, by diets which are unsuitable in a general sense in that they do not meet the general requirements of the organism. In like manner, it is possible to influence rickets by means of the diet in two ways: directly, by feeding diets having specific salt defects or defects of an unknown nature which load down the disabled mechanism governing the salt regulation of the body; and indirectly, by diets which are unsuitable for the general requirements of the organism. In both diseases the diet can increase or diminish the metabolic disturbance according as it

[16] The differences between rickets and diabetes are of course appreciated.

strains or spares the metabolism at its weak point; in both diseases the diet can increase or diminish the metabolic disturbance according as it fails or succeeds in meeting the general requirements of metabolism. In diabetes the diet is not the cause of the disease; the cause lies in an insufficiency in the internal secretion of the pancreas. So, also, in rickets the diet (considered apart from *X*) is not the cause of the disease. The cause lies in a deficiency in the regulatory influence of radiant energy or the unknown factor in the food. In both diseases, the diet exercises a profound influence, not in bringing the disease into being, but in determining the extent and character of its manifestations. It is well known that a whole variety of conditions exerts an unfavorable influence in diabetes. Among them may be mentioned infection, fatigue, lack of muscular exercise, bad hygienic surroundings, exposure to cold, mental anxiety, etc. These conditions exert their unfavorable influence in diabetes because they weigh down an organism already handicapped by a disabled mechanism. In exactly the same way confinement, infection, bad hygienic surroundings (considered apart from the absence of radiant energy), exercise a deleterious influence in rickets, further weakening an organism already suffering from a disabled metabolism. Since rickets is a disease of metabolism and the substances to be metabolized are the foods, the diet can exert a more direct influence than can conditions such as those mentioned, with the exception of infection.

The general character of the relationship of infantile tetany to rickets has become apparent, but the exact nature of the metabolic disturbance necessary for the development of tetany continues to remain obscure. Apparently the disturbance in the salt equilibrium of the blood in rickets occurs chiefly in two forms: in one the calcium is low and the inorganic phosphorus somewhere near the normal; in the other the phosphorus is low and the calcium is somewhere near the normal. Between the two are innumerable gradations. Tetany is a symptom-complex which occurs in rickets when the salt equilibrium in the blood happens to be of a kind which sets the nervous system in a state of hyper-excitability. Apparently the disturbance in the salt equilibrium in rickets which is provocative of the symptom-complex, infantile tetany, is the one characterized by low calcium and it is with the low calcium form of rickets that manifest tetany is associated. If tetany can be considered a disease, then it is the same deficiency disease as rickets; it is cured and prevented by exactly the same means and differs from rickets only in the fact that the salt equilibrium in the blood happens to assume a special form or forms.

No treatment of the etiology of rickets can be complete without a discussion of the peculiar susceptibility of the premature infant to rickets and tetany. Premature infants are usually kept in poorly ventilated rooms which are often super-heated and dark; they are closely wrapped and are placed less frequently than are children born at term, in fresh air and sunlight. These facts alone are not sufficient to explain the frequency of the occurrence of rickets. The premature infant differs from the baby born at term in several particulars. He has low basal metabolism and is able neither to generate the heat necessary for nor to maintain normal body temperature; he has little subcutaneous fat and metabolizes fat poorly; in proportion to his size he grows with great rapidity. Hamilton has made important observations on the metabolism of premature infants. During the last three months of fetal life a storage of calcium takes place in the body of the fetus and during the first few months of life after birth the infant is obliged to draw on this calcium depot. In contrast to the child born at term, the premature infant has no calcium depot (or a deficient one) and is destined to suffer from calcium-want provided calcium is not abundantly supplied in the food. The calcium which the premature infant receives in his breast milk, however, is not greatly in excess of the amount actually excreted. Hamilton's investigations suggest, therefore, that the frequent occurrence of rickets in the premature baby may be caused by a deficiency in calcium and possibly in phosphorus.

The suggestion of Hamilton may furnish part of the explanation. It seems probable, however, that the great susceptibility of the premature infant to rickets results chiefly from the immaturity of the organism and great impetus for growth. The premature infant cannot be regarded as normal and indeed does not overtake the child of the same conceptional age until five or six years have elapsed. Moreover, his growth stimulus is enormous. The premature infant must, therefore, be far more dependent on radiant energy and on *X* than the child born at term and suffers more rapidly and severely when deprived of them. The child of four is more susceptible to rickets and develops the disease more rapidly than the adult, the child of one year than the child of four, the new-born baby than the infant of one year, the premature baby than the infant born at term, and the very small premature baby than the larger premature baby. According to Yllpö very small premature infants always develop rickets. The susceptibility to rickets varies inversely, therefore, with the age and culminates in the premature infant. To state the fact in another way, the susceptibility to rickets varies directly with

the youngness of the organism or the speed of new tissue formation. A theory similar to the one just presented has been advanced by Yllpö to explain the frequency of occurrence of anemia in the new-born infant. Doubtless rickets and anemia are sister conditions and both are expression of the inability of the organism properly to discharge its metabolic functions when thrown on its own resources in an unfriendly environment without being properly prepared.

In 1906 Hansemann wrote one of the great papers on rickets, by far the greatest written on the etiology of the disease. Hansemann perceived that man in his struggle for existence against the forces of nature had created a whole fabric of abnormal conditions, and in so doing had altered his own habits and ways of living as completely as he had altered the habits and ways of living of the domestic animals which he had made dependent upon him. "Domestication," wrote Hansemann, "does not relate merely to those things which pertain to the house, as the derivation of the word might indicate, but refers to every effort on the part of man to further the survival of the race and of the individual by artificial means and to aid in the struggle against the forces of nature. By this definition it becomes at once apparent that not alone does man domesticate animals but has domesticated himself, a fact clearly recognized by Darwin." According to Hansemann, rickets is the direct result of domestication and a penalty that man pays for the artificial structure which he has created. The writers on rickets seem to have assumed that Hansemann's conception referred only to the environmental conditions of the individual; clearly his conception included also the food. The food which domesticated man eats is as far removed from the food furnished by nature than the conditions under which he lives from the natural conditions. Recent progress in the understanding of rickets has served merely to give Hansemann's conception more exact definition. Rickets is indeed a price paid by man for his abandonment of a life out-of-doors and a natural diet for a life in houses and a diet of denatured foodstuffs; it is a sign of the operation of the immutable law of nature that nothing out of accord with her shall flourish.

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